PER MANHEM

EFFECT OF CLONIDINE, METOPROLOL AND CAPTOPRIL ON THE EXERCISE INDUCED CHANGES IN THE SYMPATHETIC FUNCTION AND THE RENIN ANGIOTENSIN SYSTEM IN MAN

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Effect of clonidine, metoprolol and captopril on the exercise induced changes in the sympathetic function and the renin angiotensin system in man

Abstract

The sympathetic nervous system and the renin-angiotensin system were stimulated by bicycle ergometer exercise and inhibited by the α_2-agonist clonidine, the β_1-antagonist metoprolol and the converting enzyme inhibitor captopril, respectively, in order to explore the influence of the two systems on blood pressure regulation and the mode of action of the three different drugs. The studies were performed in patients with essential hypertension and included measurements of catecholamines, renin activity, angiotensin II and aldosterone in plasma. Similar studies but without medication were performed also in healthy males.

The studies demonstrated that the kidney as well as the heart contribute to the increase on circulating noradrenaline in connection with heavy exercise in healthy males. Clonidine reduced blood pressure (BP), pulse rate (PR), plasma noradrenaline (PNA) and plasma renin activity (PRA) before and during exercise. Metoprolol reduced BP, PR and PRA before exercise but caused no changes in plasma catecholamines. The increase in BP and PR in response to submaximal work was reduced, but the PNA response was enhanced during metoprolol. Captopril reduced BP during moderate exercise but not at near maximal work load. Captopril was without effect on the heart rate. The exercise-stimulated increase in PNA and plasma adrenaline was identical before and during captopril. The plasma concentrations of angiotensin II and aldosterone were substantially reduced, whereas PRA was considerably increased during captopril.

Long term clonidine treatment thus reduced the activity of the sympathetic nervous system as judged by plasma noradrenaline levels, whereas this was not the case with long term metoprolol treatment. Both drugs reduced the activity of the renin-angiotensin system. Short term captopril treatment had no major effect on the activity of the sympathetic nervous system. Angiotensin II seems not to play a major role for the regulation of the sympathetic nervous activity in patients with essential hypertension.

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Aldosterone, angiotensin II, captopril, catecholamines, clonidine, metoprolol, renin, sympathetic activity.

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By

Per Manhem

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This thesis is based on the following publications:

I. Manhem, P., Lecerof, H. and Hökfelt, B. (1978):
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II. Manhem, P. and Hökfelt, B. (1981):
Prolonged clonidine treatment: Catecholamines, renin activity and aldosterone following exercise in hypertensives.
Acta Med Scand 209:253-260.

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Long term treatment of moderate hypertension with the $beta_1$-receptor blocking agent metoprolol. II. Effect of submaximal work and insulin-induced hypoglycemia on plasma catecholamines and renin activity, blood pressure and pulse rate.
Eur J Clin Pharmacol 11:247-254.

V. Manhem, P., Bramnert, M., Hulthén, U.L. and Hökfelt, B. (1981):
The effect of captopril on catecholamines, renin activity, angiotensin II and aldosterone in plasma during physical exercise in hypertensive patients.
Eur J Clin Invest 11:389-395.

In the text these papers will be referred to by their Roman numerals.

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To
Ulla-Britt and Magnus

ABBREVIATIONS

CV	=	Coefficient of variation
MWC	=	Maximal working capacity
PA	=	Plasma adrenaline concentration
P-ALDO	=	Plasma aldosterone concentration
P-ANG	=	Plasma angiotensin II concentration
P-CLON	=	Plasma clonidine concentration
PNA	=	Plasma noradrenaline concentration
PRA	=	Plasma renin activity

CONTENTS

A. AIMS OF THE STUDIES 7
B. INTRODUCTION 7
CIRCULATING CATECHOLAMINES AND SYMPATHETIC ACTIVITY 8
THE RENIN-ANGIOTENSIN SYSTEM 11
THE FUNCTIONAL INTERRELATION OF THE SYMPATHETIC NERVOUS SYSTEM AND THE RENIN-ANGIOTENSIN SYSTEM 13
CLONIDINE - MODE OF ACTION 15
METOPROLOL - MODE OF ACTION 17
CAPTOPRIL - MODE OF ACTION 18
C. THE PRESENT STUDIES 20
NORMAL SUBJECTS AND PATIENTS 20
DESIGN OF THE STUDIES 22
ANALYTICAL METHODS 24
STATISTICAL EVALUATION 25
RESULTS 25
Catecholamines under Basal Conditions and Following Exercise 25
Without Medication 25
Effect of Clonidine 27
Effect of Metoprolol 29
Effect of Captopril 29
The Renin-Angiotensin System under Basal Conditions and Following Exercise 30
Without Medication 30
Effect of Clonidine 31
Effect of Metoprolol 32
Effect of Captopril 33
Blood Pressure and Pulse Rate under Basal Conditions and Following Exercise 34
Effect of Clonidine 34
Effect of Metoprolol 36
Effect of Captopril 36

DISCUSSION 37
Sympathetic Activity and Circulating Catecholamines 37
- Noradrenaline 37
- Adrenaline 41
The Renin-Angiotensin System 42
Blood Pressure and Pulse Rate 45
D. GENERAL SUMMARY AND CONCLUSIONS 47
E. ACKNOWLEDGEMENTS 51
F. REFERENCES 52

A. AIMS OF THE STUDIES

- to explore the sources of circulating catecholamines during physical exercise in man,

- to investigate the effect of long term treatment with the α-agonist clonidine and the cardioselective β-receptor blocker metoprolol, respectively, on the exercise induced increase in circulating catecholamines, renin activity and blood pressure and pulse rate in patients with essential hypertension,

- to study the effect of the converting enzyme inhibitor captopril on the renin-angiotensin system in relation to sympathetic function during exercise in patients with essential hypertension.

B. INTRODUCTION

The level of blood pressure is influenced by several factors, among them the sympathetic nervous system and the renin-angiotensin system. The activity of these systems is reflected in the plasma levels of catecholamines and renin activity/angiotensin II, respectively. The role of these factors in blood pressure regulation can be explored by stimulation and/or inhibition of the function of the respective systems. Both systems are stimulated by the assumption of upright posture and even more in connection with physical exercise. Reduced function of the two systems follows the administration of sympathetic inhibitors such as clonidine and metoprolol, acting centrally and/or peripherally. Clonidine has predominantly α_2-agonistic properties, whereas metoprolol mainly acts to block β_1-adrenoceptors. Reduced formation of angiotensin II can be obtained by the administration of the converting enzyme inhibitor captopril. The use of stimulatory procedures in combination with agents primarily interfering with the function of the sympathetic nervous system on the one hand and the renin-angiotensin system on the other also can serve to explore how these two systems are interrelated functionally. In addition, such studies might reveal the mechanisms, whereby the respective drugs act.

CIRCULATING CATECHOLAMINES AND SYMPATHETIC ACTIVITY

The present studies were concerned with noradrenaline and adrenaline. Under ordinary conditions circulating noradrenaline mainly originates from peripheral sympathetic neurons, whereas adrenaline comes from the adrenal medulla.

Noradrenaline constitutes the transmitter at most sympathetic postganglionic nerve endings. Noradrenergic neurons are also located within the central nervous system. The transmitter is stored in the synaptic knobs of adrenergic neurons in characteristic vesicles. On depolarization of the neuron, the transmitter is released from the storage sites directly into the synaptic cleft, where it interacts with a specific receptor at the postsynpatic membrane. The termination of transmitter action is complex and seems to involve several mechanisms (Iversen 1973).

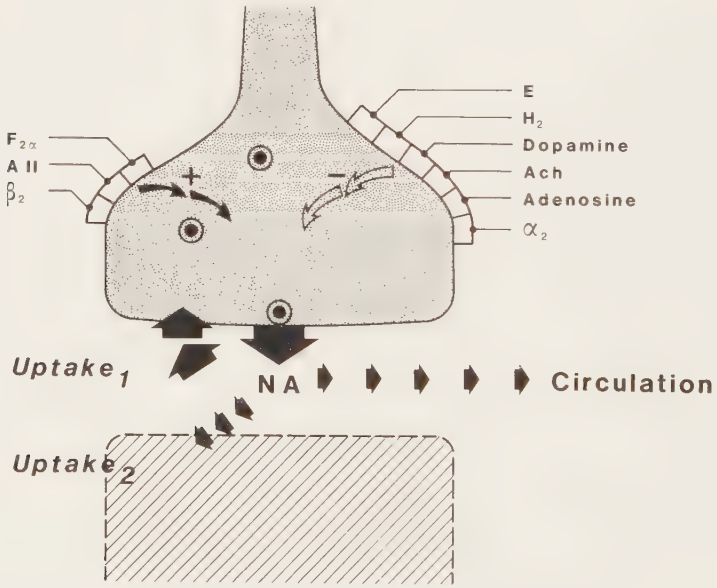

Fig. 1. Proposed mechanisms of endogenously produced substances altering output of noradrenaline from sympathetic postganglionic noradrenergic nerve terminals. For ref. see Lees 1981.

$F_{2\alpha}$ = prostaglandin $F_{2\alpha}$, E = prostaglandin E series and enkephalin/beta-endorphin, H_2 = histamine$_2$, Ach = acetylcholine.

The main part of the transmitter liberated is functionally inactivated by reuptake (uptake$_1$) into the sympathetic nerve terminal, where it can be either stored in the vesicles or degraded by monoamine oxidase (MAO) present in the neuron. At very high concentrations a small part of catecholamines is inactivated by a transport system in the extraneuronal peripheral tissues (uptake$_2$). The physiological role of this last mechanism for the removal of the transmitter has been questioned (Laverty 1978). Part of the noradrenaline released at the nerve terminals reaches the systemic circulation by diffusion and is rapidly catabolized either by catechol-O-methyltransferase (COMT) or by MAO. This inactivation and degradation of noradrenaline and other catecholamines occurs to a great extent within the liver (Hertting and LaBrosse 1962) and the metabolites are excreted either in the bile or in the urine. A fraction (2-16%) of the catecholamines circulating in the blood is excreted unchanged in the urine (Sharman 1973).

Adrenaline normally circulates in comparatively low amounts. Like noradrenaline, it can be taken up and stored by peripheral sympathetic neurons, where it will be either stored until released at a later time or degraded in a manner similar to noradrenaline (Rand et al. 1978). There is good evidence that adrenaline also can serve as a transmitter in certain central neurons (Hökfelt et al. 1974 and 1980).

The effects exerted by the sympathetic transmitters and circulating catecholamines are mediated via activation of adrenoceptors located in the cell membrane. On basis of their pharmacological properties the adrenoceptors can be divided into different types (table 1).

Table 1. Cardiovascular responses mediated by adrenoceptors (Lees 1981)

Organ affected	Receptor type	Response to stimulation
Heart	β_1, β_2	Increased automaticity
	β_1	Increased conduction velocity
	β_1	Increased excitability
	β_1	Increased force of contraction
Blood vessels	α_1, α_2	Constriction of arteries and veins
	β_1	Dilatation of coronary arteries
	β_2	Dilatation of most arteries

The classification of adrenoceptors originally developed by Ahlquist (1948) was based on the rank order of potency of a series of agonists and later on the development of specific inhibitors. Ahlquist presented evidence for at least two types of adrenoceptors, which he designated alpha and beta. Lands et al. (1967) suggested the presence of two distinct subclasses of β-adrenoceptors, which they called β_1 and β_2. Further characterization and identification have been accomplished by the use of radioligand techniques. Subsequently, two subclasses of α-adrenoceptors, α_1 and α_2, were proposed (for ref. see Starke and Endo 1976).

Plasma levels of noradrenaline are often used as an index of sympathetic nervous activity. Experimental studies demonstrate that plasma levels of noradrenaline increase, when sympathetic nerves are stimulated electrically (Haefely et al. 1965, Yamaguchi et al. 1975). Manoeuvres known to increase sympathetic activity in man such as standing and physical exercise increase the plasma levels of noradrenaline (Chodakowska et al. 1975, Christensen and Brandsborg 1973, Hansson and Hökfelt 1975). Furthermore, plasma noradrenaline correlates directly with sympathetic muscle nerve activity in normotensive man (Wallin et al. 1981). Plasma noradrenaline levels are also influenced by factors other than physical activity, e.g. sodium intake (Romoff et al. 1979), mental activity (LeBlanc et al. 1979), circadian changes (Lightman et al. 1981) and caffeine intake (Robertson et al. 1979). Accordingly, studies concerned with sympathetic activity and plasma catecholamines as far as possible should be performed under standardized conditions. Even under standardized conditions, plasma levels of noradrenaline vary markedly between subjects, partly due to individual variation in clearance and volume of distribution of catecholamines (FitzGerald et al. 1979). Direct recordings of sympathetic activity in human muscle nerves revealed pronounced differences between individuals (Sundlöf and Wallin 1977), whereas the level of sympathetic activity was remarkably constant from day to day in a given individual. These last results support earlier findings, showing that the levels of circulating catecholamines for a given individual are quite constant when measured daily over a prolonged period of time (deChamplain et al. 1976).

Noradrenaline can be converted to adrenaline under the influence of phenyl-ethanolamine-N-metyl transferase (PNMT), an enzyme present in the adrenal medulla and within certain areas in the central nervous system. Circulating adrenaline originates from the adrenal medulla. Similar to the sympathetic nervous system the activity of the adrenal medulla is influenced by a number of endogenous and exogenous factors (Nestel 1969), e.g. plasma levels increase on standing and following physical exercise. The levels also increase substantially in response to hypoglycemia.

The urinary and plasma catecholamine levels are reduced in patients with sympathetic insufficiency (Luft and von Euler 1953, Hedeland et al. 1969) and in subjects with tetraplegia devoid of central sympathetic control (Guttmann et al. 1963, Mathias et al. 1975).

The question concerning the pathogenetic role of the sympathetic nervous function in essential hypertension is still under debate. It is still not clear whether the plasma noradrenaline levels are elevated (Louis et al. 1973) or normal (Lake et al. 1977) and whether plasma catecholamines correlate with blood pressure (Louis et al. 1973) or not (Sever et al. 1977). Also, opinions vary whether they correlate with PRA (DeQuattro et al. 1977) or not (Louis et al. 1974). It seems possible that these contradictory results at least partly could be due to the fact that the studies were performed under non-standardized conditions, that different age groups or subgroups of patients with sympathetic hyperactivity were studied, etc.

THE RENIN-ANGIOTENSIN SYSTEM

Renin is a proteolytic enzyme, synthetized in the juxtaglomerular cells in the kidney. It interacts with renin substrate (angiotensinogen), synthetized by the liver, to form the decapeptide angiotensin I. This peptide is acted upon by converting enzyme to produce the octapeptide angiotensin II. Converting enzyme is present in small amounts in blood and in greater amounts in the endothels and vessels in general. Quantitatively its most important site of action is located in the lungs (Erdös 1976).

Angiotensin II is rapidly split by peptidases to form either a heptapeptide or a hexapeptide together with minor peptide fragments. The heptapeptide, also called angiotensin III, has certain angiotensin II-like properties, especially in relation to adrenal angiotensin receptors (Goodfriend and Peach 1975) mediating aldosterone secretion, whereas the hexapeptide and the minor fragments are biologically inactive.

There are four basic mechanisms engaged in the control of renin release:

- renal baroreceptors
- sodium sensitive renal receptors
- a negative intrarenal feedback system via angiotensin II
- renal sympathetic nerves and circulating catecholamines.

Angiotensin II is the most potent endogenous vasopressor agent known in man. On a molecular basis it is about 40 times more potent than noradrenaline (Douglas 1975). It induces vasoconstriction by two different mechanisms: a direct vasoconstrictor action on the vascular smooth muscle and an indirect action via activation of the sympathetic nervous system. To judge from studies with i.v. infusions of angiotensin II in man, the direct action accounts for most of the increase in total peripheral resistance underlying the pressor response (Douglas 1975). The effect of angiotensin II on the sympathetic nervous system consists of central stimulatory action to enhance sympathetic outflow and a peripheral action of angiotensin II to potentiate adrenergic transmission at peripheral neuroeffector sites (Zimmerman 1978). In experiments on various cardiac preparations, angiotensin II has also a direct positive inotropic and a weak chronotropic effect (Dempsey et al. 1971). Under experimental conditions supraphysiological doses of angiotensin II have been found to stimulate the adrenal medulla directly to release catecholamines (Peach 1971). Recently Bumpus et al. (1980) presented evidence that angiotensin could influence catecholamine release from the adrenal medulla via a central mode of action.

Angiotensin II and angiotensin III rather selectively stimulate adrenal aldosterone synthesis and secretion (Laragh 1962). In man, the i.v. infusion of non-pressor doses of angiotensin II initiates aldosterone secretion within a few minutes (Davis 1974).

Renin activity has been found in several organs in animals (Brown et al. 1967). Of special interest is its presence in the hypothalamus and other areas of the central nervous system (Ferrario et al. 1972). The physiological role of the renin angiotensin system within the central nervous system is not clear but is at present the subject of intense investigation (Unger et al. 1981). As mentioned, angiotensin II might play a role centrally as a modulator of sympathetic activity (Ferrario et al. 1972). There is some experimental evidence that the central effects of angiotensin is linked to endogenous opiates (Schaz et al. 1980). So far, no other extrarenal sites have been established for renin production but minor amounts of renin activity have been demonstrated in plasma from nephrectomized human beings (Brown et al. 1967).

The renin-angiotensin system has a potential to induce hypertension. Thus, highly elevated renin activity and severe hypertension have been a constant finding in patients with renin secreting tumours. Also, elevated plasma renin activity is commonly present in hypertension resulting from renal artery stenosis and is invarably found in malignant hypertension. In both these types of hypertension, converting enzyme inhibition reduces blood pressure (Cody et al. 1978, Oberfield et al. 1979). On the other hand, no simple relationship seems to exist between plasma renin activity and blood pressure in essential hypertension. In such patients plasma renin activity and plasma angiotensin II concentrations mostly are normal, but they may also be elevated or subnormal (for ref. see Page and Bumpus 1974, Robertson et al. 1967).

THE FUNCTIONAL INTERRELATION OF THE SYMPATHETIC NERVOUS SYSTEM AND THE RENIN-ANGIOTENSIN SYSTEM

The afferent arterioles and the juxtaglomerular cells in the kidney are highly innervated by sympathetic nerves and there is good evidence that the sympathetic nervous system plays a profound role for the regulation of renin secretion (Ganong 1972, Hökfelt et al. 1975, Reid et al. 1978).

Increased sympathetic nervous activity seems to stimulate renin secretion either directly via an action on the juxtaglomerular cells or indirectly via constriction of the afferent arterioles (Reid et al. 1978). It is still not fully clear whether this is mediated via α- or β-adrenergic receptors, but most studies support the view that the catecholamine-induced renin release mainly is mediated via β-receptors (Assaykeen et al. 1970).

Experimental data demonstrate that angiotensin II can act both centrally and peripherally to modulate sympathetic function, thus indicating the possibility of a reciprocal interrelationship between the sympathetic nervous system and the renin-angiotensin system.

With respect to the central effect of angiotensin II on sympathetic function, Bickerton and Buckley (1961) demonstrated a hypertensive response in dogs by means of cross-circulationg experiments. Furthermore, small amounts of angiotensin II infused in the vertebral arteries in animals and man, lead to a rise in systemic blood pressure (for ref. see Ferrario et al. 1972). The responsive region appears to be located in the area postrema of the medulla but also other areas of the brain devoid of blood-brain barrier might be involved.

At the peripheral site angiotensin II has been found to facilitate ganglionic transmission (Douglas et al. 1967) but this effect is variable (Elijovich and Krakoff 1980). A more consistent response to angiotensin, even in low dosages, consists of facilitation of neurotransmission at the sympathetic nerve endings (Zimmerman 1978). Several mechanisms have been suggested, such as increased noradrenaline biosynthesis in the adrenergic terminals, depressed reuptake of catecholamines and increased noradrenaline output.

The contribution of central and peripheral adrenergic facilitation by angiotensin to sympathetic function as a whole has not been clearly established, neither under physiological conditions nor in different forms of hypertension in man. Experimental studies suggest that the peripheral adrenergic facilitation contributes to the induction of renovascular hypertension in the rabbit (Ichikawa et al. 1978) and that central adrenergic facilitation might be in-

volved in spontaneous hypertension in the rat (for ref. see Brody et al. 1980). The introduction of angiotensin antagonists and converting enzyme inhibitors seems to open new avenues for the clarification of these questions (Zimmerman 1981).

CLONIDINE - MODE OF ACTION

Clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride) was originally designed as a nasal decongestant agent but was soon discovered to possess a potent blood pressure lowering effect in man (Graubner and Wolf 1966) It then became clear that clonidine, although an α-adrenoceptor agonist, lowers blood pressure via an action on sympatho-inhibitory α-receptors in the brain stem (Kobinger and Walland 1967, Schmitt et al. 1967). This discovery pointed to the possibility that α-receptors play an important role in blood pressure regulation and initiated intense research in this field.

Central α-receptors are engaged in the baroreflex control of blood pressure and are located not only in the brain stem but also in higher centers, where they serve to modulate the baroreflexes. Stimulation of these receptors, for instance by clonidine, diminishes sympathetic outflow from the central nervous system and increases parasympathetic outflow to the heart via the vagal nerve.

As to the more precise mechanism, Schmitt et al. (1971) demonstrated that the central hypotensive effect of clonidine is diminished or even abolished by various α-sympatholytic agents. Haeusler (1974) found no proof for a central presynaptic mode of action to explain the inhibitory effect of sympathetic nerve activity. These observations led Haeusler to the conclusion that clonidine mainly acts as an α-adrenoceptor agonist to stimulate central postsynaptic α-adrenoceptors resulting in reduced peripheral sympathetic activity. Haeusler suggested that this might involve stimulation of inhibitory neurons located in the region of nucleus tractus solitarii.

Alternative mechanisms for the central action of clonidine have, however, been proposed. In peripheral organs, such as the isolated pulmonary artery in the rabbit, clonidine was found to possess a stimulatory effect on presynaptic α-adrenoceptors (α_2) leading to an inhibition of the release of endogenous noradrenaline (Starke et al. 1975). Extrapolation of this finding to central α-adrenoceptors leads to the assumption that clonidine inhibits the release of noradrenaline in the brain stem.

Moderate dosages of clonidine, given to tetraplegic subjects devoid of central sympathetic control, reduce the urinary bladder stimulated increase in blood pressure and plasma noradrenaline (Mathias et al. 1979). Thus, clonidine might also stimulate presynaptically located α_2-adrenoceptors of the peripheral sympathetic nervous system inhibiting the release of noradrenaline. However, this mechanism of action is of minor importance for the reduction of blood pressure in patients with essential hypertension (Reid 1981).

Clonidine has also been demonstrated to stimulate histamine H_2-receptors in animals both centrally (Audigier et al. 1976) and peripherally (Parsons 1978). Karppanen et al. (1976) reported that clonidine given intravenously to rats in a dose inducing marked hypotension, could be abolished by intracerebroventricular pretreatment with the selective H_2-receptor antagonist metiamide. These findings raise the question whether central H_2-receptors might be engaged in the hypotensive effect of clonidine. Peripheral histamine H_2-receptors seem, however, not to be involved in the circulatory effects of clonidine in man (Watkins et al. 1980).

The possibility that clonidine might interact also with central opiate receptors was raised following its successful use in the management of the narcotic withdrawal syndrome (Gold et al. 1978). Endorphines administered centrally or peripherally in animals lower blood pressure (Laubie et al. 1977). At least short-term circulatory effects of i.v. clonidine given to normotensive humans seem, however, not to be mediated by opiate receptors (Watkins et al. 1980). There are also reports indicating that clonidine might depress sympathetic activity by stimulating inhibitory 5-HT-receptors located on sympathetic preganglionic neurons (Franz et al. 1978, Scriabine 1979).

In summary, there is good evidence that clonidine stimulates central α-adrenoceptors in the brain stem leading to diminished peripheral sympathetic activity and as a consequence a decrease in arterial blood pressure and heart rate. It seems possible that the inhibitory effect of clonidine on sympathetic activity is to some extent exerted via peripheral presynaptic α-receptors. Stimulation of central α-adrenoceptors also facilitates vagally mediated reflexes. Also, other types of receptors might be engaged in the mechanisms mediating the hypotensive effect of clonidine.

METOPROLOL - MODE OF ACTION

Despite the fact that β-adrenergic blocking agents have gained wide acceptance as antihypertensive drugs, the mechanism for their antihypertensive effect still remains unclear.

Metoprolol (1-isopropylamino-3-(4-(2-metoxyethyl)-phenoxy)-2-propanol) inhibits the cardiac effects of isoprenaline in cats at doses much lower than those required to inhibit the vasodilator response (Åblad et al. 1973) and is devoid of intrinsic β-adrenoceptor agonist activity (Åblad et al. 1973). This and other findings (Åblad et al. 1974) suggest that metoprolol has little effect on the β-adrenoceptor mediated peripheral vasodilator effect of isoprenaline or adrenaline (cardioselective = β_1).

Acute or chronic administration of metoprolol to normal or hypertensive patients results in a dose related reduction of heart rate and blood pressure (Johnson 1975, Hansson et al. 1977). Lund-Johansen and Ohm (1976) reported a 24% reduction of the heart rate and a 13% decrease in the mean arterial pressure in patients with essential hypertension treated with metoprolol for one year. Metoprolol reduces plasma renin activity in patients with moderate hypertension (Hansson et al. 1977). Thus, the antihypertensive effect of metoprolol seems to be related to the ability to block β-adrenergic receptors located in the kidney as well as in the heart.

A number of animal studies suggests that β-blockers in higher dosages exert an additional antihypertensive action through a decrease in sympathetic nerve activity via either a central or a peripheral mode of action (Sweet and Wenger 1976, Saelens et al. 1977). However, in man plasma noradrenaline levels have been reported to increase (Rahn et al. 1978), decrease (Brecht et al. 1976) or not to change (Irving et al. 1974) following treatment with β-blockers. Recently, Jackson and Campbell (1981) suggested that propranolol antagonizes angiotensin II enhancement of sympathetic nerve stimulation by increasing prostaglandin levels in vascular tissue. So far, no similar studies concerning the action of metoprolol were published.

CAPTOPRIL - MODE OF ACTION

Captopril (SQ 14.225) (D-3-mercapto-2-methylpropranoyl-L-proline) is a potent, selective inhibitor of the angiotensin-converting enzyme, which is identical to kininase II. This enzyme has a key-role both in the renin-angiotensin system and the kallikrein-kinin system (fig. 2). Furthermore, it has some properties in common with the enzymes degrading enkephalins (Sullivan et al. 1980, Benuck et al. 1981).

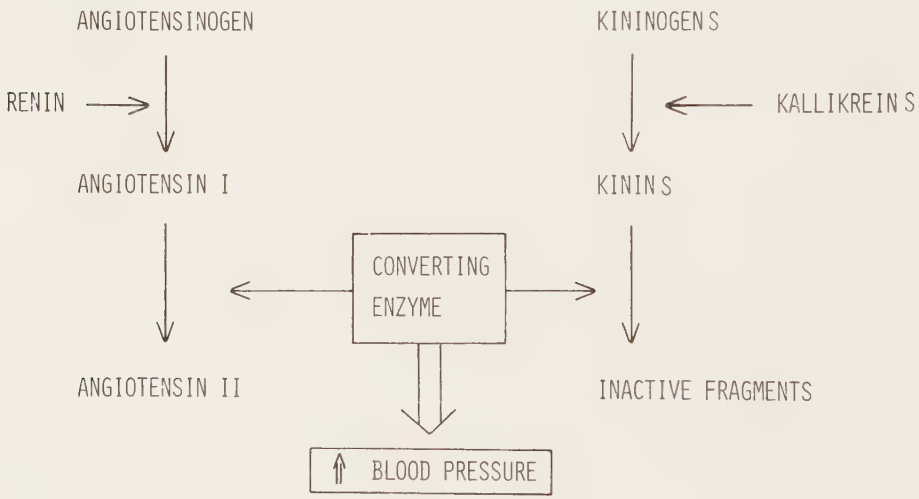

Fig. 2. Illustration of the key-role of converting enzyme in blood pressure regulation.

The importance of the activity of the renin-angiotensin system for the antihypertensive action of captopril is not clear. Thus, captopril effectively reduces blood pressure in most forms of hypertension, including human renal hypertension and essential hypertension, both with low, normal and high plasma renin activity (for ref. see Antonaccio et al. 1981). In patients with renin-dependent hypertension, such as cases with renal artery stenosis, captopril has a marked hypotensive effect, and the same is true in salt-depleted patients with essential hypertension (Brunner et al. 1978, 1979). These and many other studies seem to demonstrate an important role of the renin-angiotensin system for the hypotensive action of captopril. In fact, a close correlation has been found between the blood pressure response to the drug and the initial plasma renin activity (Case et al. 1980). However, as mentioned, there are studies demonstrating a hypotensive effect of captopril also in low renin hypertension (Bengis et al. 1978) as well as in nephrectomized patients (Man in't Veld et al. 1979). Thus, it appears that captopril in certain cases lowers blood pressure via a mechanism independent of circulating angiotensin II. One possible mode of action could be the potentiation of the direct effects of bradykinin due to inhibition of bradykinin inactivation. As bradykinin increases the formation and release of renal prostaglandins and since this effect is enhanced by captopril, it has been suggested that this might be one mechanism for the antihypertensive action of captopril (Murthy et al. 1978, Moncada et al. 1979). However, contradictory findings concerning kinins and prostaglandins were reported by Hulthén (1980). Recently, Garst et al. (1979) on basis of studies in the rat suggested that the vascular renin angiotensin is of importance for the maintenance of hypertension. Similarly, Antonaccio et al. (1981) presented evidence that captopril might decrease blood pressure in normal or low reninemic hypertension by inhibiting the vascular renin-angiotensin system.

Captopril does not readily enter the central nervous system (Rubin et al. 1978). Thus, it seems less likely that it acts to lower blood pressure via a direct action on the brain renin-angiotensin system (Suzuki et al. 1981).

Although the hypotensive effect of captopril is linked to a reduction of systemic vascular resistance, no consistent effect on heart rate has been found in most studies (Heel et al. 1980). The lack of reflex-induced tachycardia is compatible with an inhibitory effect on the sympathetic nervous function. Although Antonaccio and Kerwin (1981) demonstrated a selective inhibitory effect

on the vascular part of the sympathetic nervous system in spontaneously hypertensive rats, no such effect was found for the cardiac part. Also, captopril induced no reduction of plasma noradrenaline under basal conditions in patients with essential hypertension (Bravo and Tarazi 1979, de Bruyn et al. 1979, Waeber et al. 1980). Thus, so far no definite conclusion could be drawn regarding the effect of captopril on the activity of the sympathetic nervous system.

C. THE PRESENT STUDIES

NORMAL SUBJECTS AND PATIENTS

In an attempt to identify the sources of circulating catecholamines, six healthy males, aged 23-35 years, were studied in connection with physical exercise (I).

The remaining four studies were performed in patients with essential hypertension. They were selected on an ambulatory basis after having repeatedly presented a blood pressure no less than 160/95 mmHg in the supine position after a minimum of 20 min rest. Before acceptance each patient was subjected to special investigations in order to exclude the presence of secondary hypertension.

Seven men (33-55 years) and one woman (49 years) participated in the clonidine studies (II, III). Detailed data are given in II.

The metoprolol studies (IV) included nine men (31-52 years). Clinical and laboratory findings were summarized in table 2. In retrospect, one patient (no II) was found to have hereditary polycystic renal disease in spite of presenting normal pyelography and radiorenography at the initial investigations.

Captopril was studied in seven males (35-58 years). Detailed data are given in V.

Table 2. Clinical and laboratory findings in 9 patients with hypertension, as registered during hospitalization (IV)

No	Age	Body weight kg	Blood pressure (mean) mmHg	Eye ground KW	Cardiac size ml/m^2	ECG	Serum creatinine µmol/l	Serum potassium mmol/l
I	47	74	155/103	I-II	460	LVH+	110	3.6
II	38	72	140/101	0	340	LVH+	100	3.9
III	31	91	142/107	0	590	LVH(+)	120	3.7
IV	44	82	154/111	0-I	500	LVH(+)	100	4.2
V	38	85	139/96	0-I	455	Normal	110	4.0
VI	51	89	145/103	I	400	LVH(+)	90	3.9
VII	45	74	148/101	II	390	Normal	110	4.1
VIII	50	74	152/93	0	350	Normal	100	4.0
IX	52	87	152/108	II	430	LVH(+)	100	3.9

DESIGN OF THE STUDIES

As previously stated, one principal aim of the present studies was to explore the effect of three different kinds of antihypertensive agents on the sympathetic nervous function and the renin-angiotensin system. Physical exercise was applied in order to activate the two respective systems. This procedure was adopted in order to amplify even minor effects of the agents. Studies of this kind are also of importance from therapeutic point of view. In order to identify the sources of circulating catecholamines in connection with physical exercise, a special study was designed, in which blood samples were collected at various levels within the circulatory system. Plasma clonidine concentrations were measured to study their correlations to the effect of the drug clinically and on catecholamines and renin activity.

The studies with clonidine, metoprolol and captopril were performed on a metabolic ward with defined sodium and potassium intake. Each patient was subjected to special studies (see below) before and during medication. In the metoprolol study identical investigations were performed on placebo. The studies on the sources for catecholamines in healthy males were performed on an ambulatory basis.

The following special investigations were performed before and during medication:

Clonidine:
Blood pressure, pulse rate, PNA, PA, PRA and P-ALDO were measured in the supine and the upright position and also in connection with submaximal exercise.

Metoprolol:
Blood pressure, pulse rate, PNA, PA and PRA were determined in connection with submaximal exercise.

Captopril:
Blood pressure, heart rate, PNA, PA, PRA, P-ANG and P-ALDO were determined in connection with submaximal exercise.

Clonidine was administered four times daily, starting with 200 µg per day for four weeks, followed by 300-600 µg daily for another 4-16 weeks and continued during the second hospitalization (II). Before treatment and at the end of each treatment period P-CLON was repeatedly measured during one dosage interval and related to PNA, PA, PRA and blood pressure and pulse rate (III). In the metoprolol studies placebo tablets were given, half a tablet three times daily for four weeks. Treatment with metoprolol was initiated by the administration of 75 mg daily and then gradually increased. The final dosage was 150-450 mg daily, which was maintained for 4-17 weeks (IV). In the captopril study the drug was administered three times daily from day 5 in stepwise increasing dosages from 75 mg daily to a total dose of 600 mg per day on days 9-11 (V).

In the patients a standardized submaximal work test was applied for 6 min (IV) or 20 min (II, V) with the patients seated on an electrically braked variable load bicycle ergometer (Siemens-Elema). The work load used was determined in advance for each patient without medication. In the clonidine study (II) a work load corresponding to 80% of estimated maximal work capacity (Åstrand 1960) was used. In the metoprolol study (IV) the patients were subjected to a work load causing a steady state pulse rate of approximately 130/min. In the captopril study (V) a work load of 40% of MWC was applied for the first 10 min, immediately followed by a work load of 70% of MWC for another 10 min.

Systolic and diastolic (Korotkoff phase 4) blood pressure was regularly recorded by one and the same person in the respective studies. Pulse rate was determined either by continuous electrocardiography (V) or by palpation of the radial pulse. Blood was drawn from an antecubital vein before and immediately after the work test. In the clonidine study (II) additional samples were drawn every fifth minute during the test and in the captopril study (V) also after 10 min bicycling. The plasma samples were assayed for PNA (II, IV and V), PA (II, IV and V), PRA (II, IV and V), PA II (V) and P-ALDO (II and V).

The six male volunteers (I) were subjected to physical exercise in the supine position after 30 min of rest, starting with a work load of 50 W for 9 min followed by 150 W for another 9 min. PNA and PA were determined in blood samples from the brachial vein, the brachial artery, the left renal vein and the femoral artery. In three of the subjects catecholamines were determined also in blood collected from the coronary sinus. All samples were taken

simultaneously firstly in the supine position after 30 min of rest and then in connection with exercise at the end of the two different work loads.

ANALYTICAL METHODS

Plasma catecholamines: Double isotope derivative technique (Engelman and Portnoy 1970) (I, II, III, IV).

Single isotope radioenzymatic assay (Peuler and Johnson 1977) (V).

Plasma renin activity: Radioimmunoassay (Haber et al. 1969, Cohen et al. 1971).

Plasma angiotensin II: Radioimmunoassay (Kappelgaard et al. 1976).

Plasma aldosterone: Radioimmunoassay according to Ito et al. (1972) (II) and according to Ogihara et al. (1977) (V).

Plasma clonidine: Gas chromatography (Edlund 1980).

The plasma levels of noradrenaline, adrenaline, aldosterone and clonidine were expressed in nmol/l, plasma renin activity in μg/l/3 h and plasma angiotensin II in pg/ml.

Intra- and interassay coefficient of variation for the respective analytical procedures used, except P-CLON, were presented in II and V. Details concerning P-CLON determinations were given in III.

STATISTICAL EVALUATION

Statistical analysis was performed by non-parametric methods in studies II and V. Mann-Whitney´s (Wilcoxon) rank sum test, Wilcoxon´s two-sample test and Spearman´s rank-correlation (R) were used. Statistical significance of differences was assessed by parametric methods in studies I, III and IV, where Student´s t-test for paired data and conventional coefficient of correlation (r) were used. The level of significance was taken as $p < 0.05$. Values are given as mean ± SE. * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$.

RESULTS

As shown in the metoprolol study (IV), placebo treatment had no effect on any of the parameters studied. In the following only values before and during medication are given.

Catecholamines under Basal and Stimulated Conditions

Without Medication

In healthy males (n = 6) the PNA found at different levels in the arterial and venous vascular system showed no significant variation when measured simultaneously under resting conditions in the supine position (I). However, the lowest value was registered in plasma from the brachial artery (1.3 ± 0.2) and the femoral vein (1.3 ± 0.2), whereas the highest value was found in the brachial vein (2.1 ± 0.5). In the left renal vein PNA was 1.8 ± 0.4 and in the coronary sinus (n = 3) 1.4 ± 0.2. PA determined simultaneously with PNA showed no significant differences, the absolute values ranging 0.14-0.82. Brachial vein PA under resting conditions was 0.5 ± 0.08.

In the clonidine study (II) PNA was 1.9 ± 0.2 and PA 0.3 ± 0.02 in samples taken from an antecubital vein under resting conditions in supine position. For the patients in the metoprolol study (IV) the corresponding values were 2.3 ± 0.4 for PNA and 0.3 ± 0.04 for PA (Hansson et al. 1977).

In the hypertensive patients, plasma catecholamines increased rather markedly in the standing position. Thus, in the clonidine study, PNA as measured in peripheral vein was 4.8 ± 0.7 in the upright position as compared to 1.9 ± 0.2 in the supine position ($p < 0.05$). PA increased in a similar pattern (from 0.3 ± 0.02 to 0.6 ± 0.10, $p < 0.05$). In the metoprolol series, PNA increased from 2.3 ± 0.4 to 7.1 ± 1.1 ($p < 0.001$) and PA from 0.3 ± 0.04 to 0.6 ± 0.08 ($p < 0.001$).

Physical exercise resulted in a significant increase in the plasma catecholamines when measured at various locations in healthy individuals, particularly in the heart and in the kidney. Thus, at a work load of 50 W PNA increased signficantly in the brachial artery, the left renal vein and the femoral vein, whereas for PA a significant increase was found only in the femoral vein. When the work load was increased to 150 W, PNA in the left renal vein significantly exceeded the concentration in the brachial vein, the brachial artery and the femoral vein. At the heavier work load, the PNA in the coronary sinus reached the same levels as in the left renal vein. During resting conditions, no significant AV-differences were found for PNA and PA, neither for the heart nor for the kidney. Using an estimated figure for the coronary and kidney blood flow, respectively, (Andersen 1968) the net output of noradrenaline was calculated according to the formula:

net output = AV-difference PNA x plasma flow.

Using this formula the net output of noradrenaline was 2.25 nmol/min from the heart and 0.36 nmol/min from the kidney at a work load of 150 W.

In response to physical exercise in the sitting position, PNA increased considerably before medication in all hypertensive patients.

	Prior to clonidine	Prior to metoprolol	Prior to captopril
PNA before exercise	4.3 ± 0.8	7.1 ± 1.1	2.9 ± 0.5
PNA after exercise	10.0* ± 1.5	11.1* ± 1.5	10.4** ± 2.0

PA increased in response to physical exercise in the clonidine (II) and captopril (V) studies, whereas no significant increase was registered in the metoprolol study (IV), in which the work load also was considerably lower.

	Prior to clonidine	Prior to metoprolol	Prior to captopril
PA before exercise	0.4 ± 0.03	0.7 ± 0.07	0.5 ± 0.04
PA after exercise	0.8* ± 0.10	1.0 ± 0.14	1.2** ± 0.08

Effect of Clonidine

Long term clonidine medication significantly lowered peripheral vein PNA both in the supine and in the upright position. Clonidine caused no changes in supine PA but the response to standing was reduced.

	Prior to clonidine	During clonidine
PNA supine	1.9 ± 0.2	0.9* ± 0.1
PNA upright	4.8 ± 0.7	3.1* ± 0.7
PA supine	0.2 ± 0.05	0.2 ± 0.04
PA upright	0.6 ± 0.10	0.4* ± 0.10

Steady state plasma clonidine concentration immediately before the next dose was 1.4 ± 0.1 on medication with 200 µg daily and 4.1 ± 0.6 on 506 ± 49 µg daily. The relation between the plasma levels of clonidine and catecholamines in the supine position was calculated at both clonidine dosages. The logarithm of P-CLON was directly related to the percentage reduction of PNA ($r = 0.67$, $p < 0.01$) (fig. 3), but not to PA.

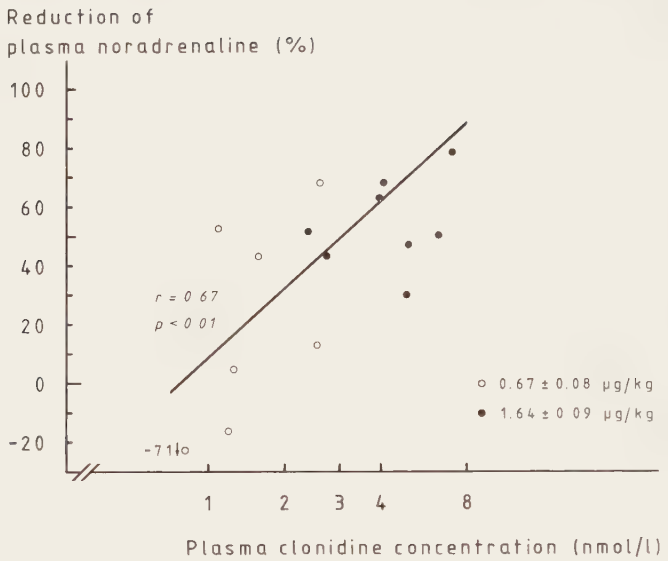

Fig. 3. Reduction of supine plasma noradrenaline in relation to plasma clonidine during chronic treatment with two different dosages.

PNA increased in response to exercise also during clonidine medication, but all levels were lower than before clonidine (fig. 4). A similar pattern was seen for PA (fig. 4).

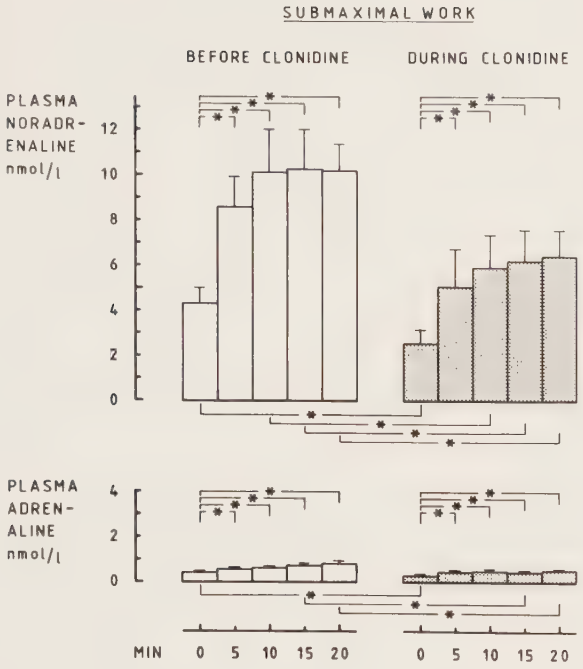

Fig. 4. Plasma catecholamines in connection with submaximal exercise before and during prolonged clonidine treatment.

Effect of Metoprolol

The PNA response to exercise was more pronounced during metoprolol (from 6.2 ± 0.8 to 17.2 ± 2.9) as compared to before medication (from 7.1 ± 1.1 to 11.1 ± 1.5 ($p < 0.05$), whereas the adrenaline response showed no significant differences.

Effect of Captopril

During captopril PNA and PA increased in response to exercise in a similar way as compared to before medication.

	Before captopril			During captopril		
Min	0	10	20	0	10	20
PNA	2.9 ±0.5	4.2** ±0.6	10.4** ±2.0	2.2 ±0.3	4.1** ±0.7	11.6** ±2.1
PA	0.5 ±0.04	0.6 ±0.07	1.2** ±0.08	0.4 ±0.03	0.6** ±0.06	1.4** ±0.09

The Renin-Angiotensin System under Basal and Stimulated Conditions

Without Medication

PRA increased significantly in the upright as compared to the supine position, both in the clonidine (II) and the metoprolol (Hansson et al. 1977) series:

	Prior to clonidine	Prior to metoprolol
PRA supine	3.8 ± 0.9	1.8 ± 0.4
PRA upright	8.6* ± 0.9	4.5* ± 1.1

Also P-ALDO, as measured in the clonidine series, increased significantly on standing (from 0.4 ± 0.1 to 0.9 ± 0.2, $p < 0.05$).

In response to physical exercise, PRA increased significantly in all three patient groups. Similarly, P-ALDO increased significantly after exercise when measured prior to treatment with clonidine and captopril, respectively. P-ANG was measured only in the captopril group, where a significant increase was found after exercise.

	Prior to clonidine	Prior to metoprolol	Prior to captopril
PRA before exercise	7.9 ± 1.6	4.6 ± 1.4	4.2 ± 1.4
PRA after exercise	13.5* ± 2.8	8.7* ± 2.1	7.3* ± 2.1
P-ALDO before exercise	0.7 ± 0.2		0.7 ± 0.1
P-ALDO after exercise	1.4* ± 0.4		1.0** ± 0.2
P-ANG before exercise			20 ± 5
P-ANG after exercise			38** ± 9

Effect of Clonidine

Long term medication with clonidine 506 ± 49 µg/day lowered peripheral vein PRA in the supine position from 3.8 ± 0.9 to 1.6 ± 1.1 ($p < 0.05$) and in the upright position from 8.6 ± 0.9 to 3.8 ± 0.7 ($p < 0.05$). Clonidine had no significant effect on P-ALDO either in the supine or in the upright position. When calculated at the two dosages of clonidine (200 µg/day and 506 ± 49 µg/day) no significant relationship was found between P-CLON and PRA in the supine position for the whole group of patients. However, in six of the eight patients clonidine reduced PRA markedly and in these six patients a significant relationship was found between the reduction (per cent) of PRA and the logarithm of P-CLON ($r = 0.91$, $p < 0.001$).

The exercise induced increase in PRA and P-ALDO, respectively, was significantly reduced during clonidine treatment with 506 ± 49 µg/day (fig. 5).

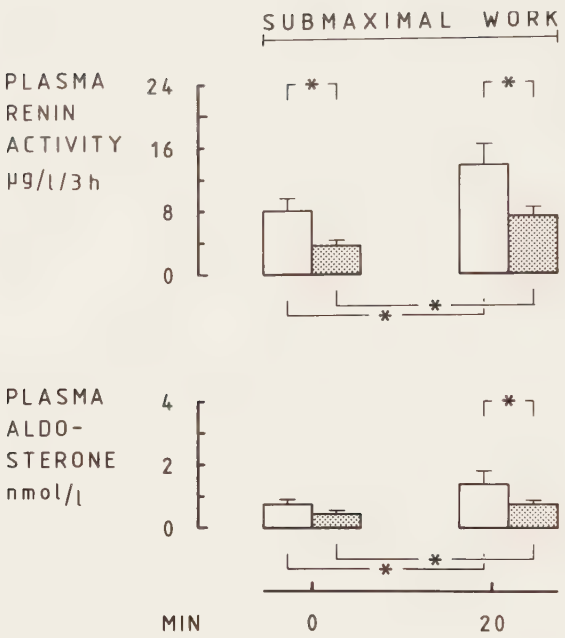

Fig. 5. PRA and P-ALDO before and after submaximal exercise before and during prolonged clonidine treatment (open columns, control; stippled columns, clonidine).

Effect of Metoprolol

Treatment with metoprolol resulted in a significant decrease in PRA both in the supine (from 1.9 ± 0.4 to 1.0 ± 0.4, $p < 0.001$) and the upright position (from 4.6 ± 1.4 to 2.5 ± 1.0, $p < 0.05$) (Hansson et al. 1977).

Similarly, metoprolol reduced the pre-exercise PRA values from 4.6 ± 1.4 to 2.5 ± 1.0 ($p < 0.05$). Following exercise, on the other hand, the PRA response varied from one patient to another and no significant difference was found in the group as a whole (PRA before metoprolol 8.7 ± 2.1, during metoprolol 6.9 ± 2.6, $p > 0.05$).

Effect of Captopril

Captopril treatment as such significantly reduced P-ANG and P-ALDO but increased PRA when measured before exercise (fig. 6). In response to exercise, both PRA, P-ANG and P-ALDO showed an increase (fig. 6).

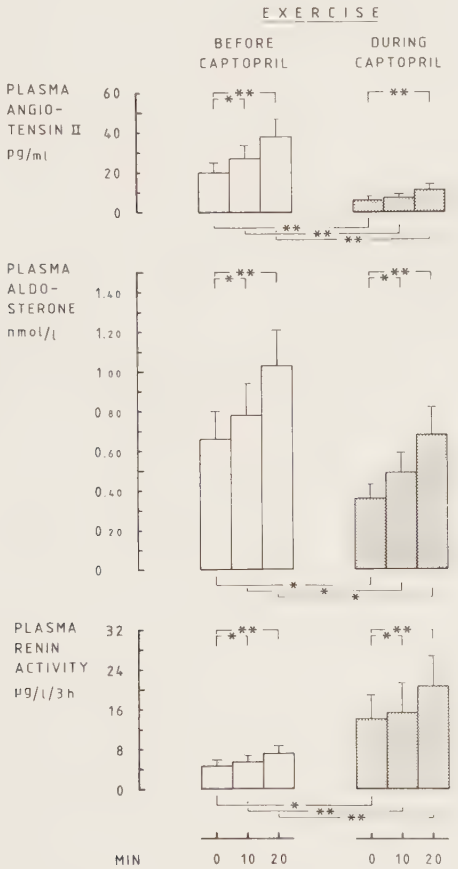

Fig. 6. The response of P-ANG, P-ALDO and PRA to graded bicycle ergometer exercise before medication and following seven days of captopril treatment.

Blood Pressure and Pulse Rate under Basal Conditions and Following Physical Exercise

Effect of Clonidine

Clonidine reduced systolic and diastolic blood pressure and also pulse rate in the supine and the upright position (fig. 7).

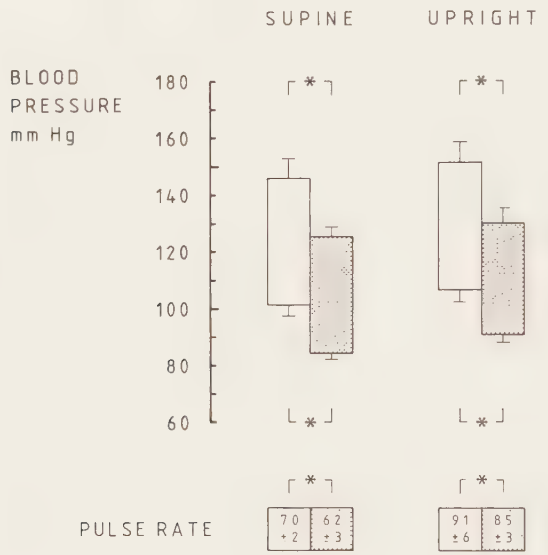

Fig. 7. Blood pressure and pulse rate in the supine and the upright position before and during prolonged clonidine treatment (506 ± 49 µg/day). Open columns, control; stippled columns, clonidine.

The clonidine induced decrease in diastolic blood pressure was positively correlated ($R = 0.76$, $p < 0.05$) to the percentage decrease in PNA in the supine position and the absolute decrease in PNA ($R = 0.80$, $p < 0.05$) in the upright position. Before medication, pulse rate was positively correlated to PNA ($R = 0.84$, $p < 0.05$) in the supine position. Blood pressure and pulse rate

were reduced by clonidine before and during submaximal work (fig. 8). Before medication the increase in pulse rate in response to exercise was positively correlated to the increase in PNA (min 10, $R = 0.73$, $p < 0.05$; min 15, $R = 0.79$, $p < 0.05$; min 20, $R = 0.74$, $p < 0.05$).

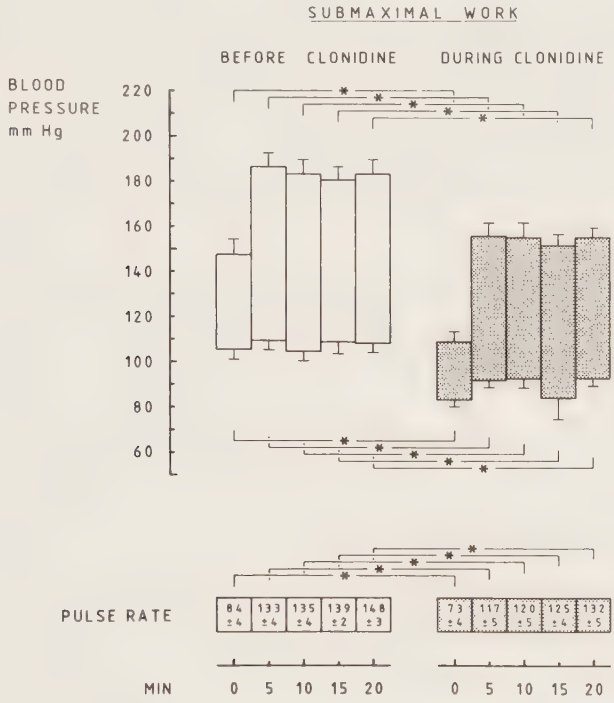

Fig. 8. Blood pressure and pulse rate in connection with submaximal exercise before and during prolonged clonidine treatment (506 ± 49 µg/day).

The relation of P-CLON to blood pressure and pulse rate in the supine position was calculated at two dose levels (200 µg daily and 506 ± 49 µg daily). Three patients showed no further decrease in mean arterial pressure with an increase of plasma clonidine levels. However, in five patients a linear relationship with a significant slope ($r = 0.91$, $p < 0.001$) was found between the mean arterial blood pressure reduction and P-CLON. No relationship was found between P-CLON and pulse rate reduction.

Effect of Metoprolol

Metoprolol reduced systolic and diastolic blood pressure and pulse rate before and after submaximal exercise.

	Prior to metoprolol	During metoprolol
Blood pressure		
before exercise	139 ± 3/97 ± 2	115** ± 4/81** ± 3
after exercise	186 ± 6/99 ± 2	147** ± 6/83** ± 3
Pulse rate		
before exercise	89 ± 4	63*** ± 3
after exercise	132 ± 3	98*** ± 4

Effect of Captopril

Captopril lowered systolic and diastolic blood pressure before and during exercise at a work load of 40% of MWC (fig. 9). At 70% of MWC systolic blood pressure was significantly reduced only after 4 minutes, and diastolic blood pressure after 2, 4 and 6 minutes of exercise. However, only six of the nine patients were able to continue exercising beyond 6 minutes on this work load, both before and during medication.

Before exercise a positive correlation was recorded between the captopril induced decrease in diastolic blood pressure and PRA before medication ($R = 0.74$, $p < 0.05$). The decrease in diastolic blood pressure was also significantly related to the captopril induced increase in PRA ($R = 0.82$, $p < 0.05$) and the decrease in P-ALDO ($R = 0.73$, $p < 0.05$) but not to the decrease in P-ANG ($R = 0.66$, $0.1 > p > 0.05$).

Before captopril, a direct relationship was registered between the exercise induced increase in systolic blood pressure (difference between the pre-exercise value and that after 20 min of exercise) and the change in PNA ($R = 0.86$, $p < 0.01$).

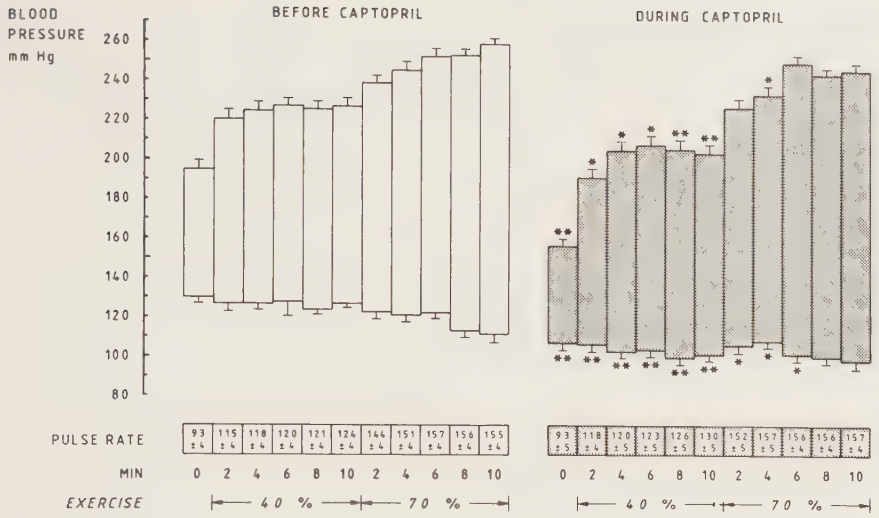

Fig. 9. Blood pressure and pulse rate in connection with submaximal exercise. The work load was for the first 10 min 40% of MWC and for the last 10 min 70% of MWC.

DISCUSSION

Sympathetic Activity and Circulating Catecholamines

Noradrenaline. There is general agreement that circulating noradrenaline to a great extent originates from the peripheral sympathetic nerve endings but so far very few studies were designed to define more specifically its tissue sources in man. Indirect evidence for the heart as one source was reported by DeQuattro and Chan (1972) and Christensen and Brandsborg (1973) on basis of a positive correlation between the pulse rate and the peripheral plasma levels of noradrenaline. Our findings in healthy male volunteers (I) clearly demonstrate that not only the heart but also the kidneys contribute to the plasma noradrenaline content at least in connection with physical exercise. A net cardiac output of noradrenaline following exercise in patients with heart disease was reported by Miura et al. (1976). Further support for the kidney as a noradrenaline source was recently reported in cirrhotic patients with in-

creased sympathetic activity (Henriksen et al. 1981) and in patients with hypertension (Brown et al. 1981).

In our studies resting plasma noradrenaline tended to be higher in the antecubital vein as compared to the brachial artery. A significant difference was earlier reported by Vendsalu (1960) in resting, normotensive men and by Halter et al. (1980) in subjects undergoing elective dental surgery. These results indicate a net release of noradrenaline, although minor, from the sympathetic nerve endings in the upper extremities. A close correlation did, however, exist between arterial and venous noradrenaline both in the present studies and those reported by Halter et al. (1980) and Watson et al. (1979).

PNA increased stepwise in response to upright posture and physical exercise. These manoeuvres are known to stimulate the sympathetic nervous system and the results support the concept that PNA can serve as an index of sympathetic activity. The finding by Wallin et al. (1981) that the plasma levels of noradrenaline at rest were related to the activity of sympathetic muscle nerves when recorded directly gives further support to this concept.

Hökfelt et al. (1975) and Wing et al. (1977) demonstrated that short term as well as acute administration of clonidine in hypertensive and normotensive man is associated with reduced levels of circulating noradrenaline. The present study demonstrates that suppression of noradrenaline production follows also long term clonidine treatment. Furthermore, the finding of a significant correlation between the reduction of PNA and the plasma clonidine concentration supports the view of a dose dependent reduction of the sympathetic nervous activity. Cohen et al. (1981) treated a similar patient material with clonidine for one month and measured the urinary excretion of catecholamines, metanephrines and VMA, applied the cold pressor test and studied the effect of phentolamine on blood pressure. However, they found no evidence for changes in sympathetic tone. These seemingly conflicting results are not readily explained. However, the studies by Cohen et al. were performed under less standardized conditions, the mean dosages of clonidine were somewhat lower (400 as compared to 506 µg/day) and clonidine was administered only twice a day as compared to four times a day. Furthermore, in the present study the percentage decrease in PNA was positively correlated to the clonidine induced decrease in diastolic blood pressure in supine position. This seems to indicate that inhibition of the sympathetic activity is an essential part of the antihypertensive effect of

clonidine. Reduced levels of plasma noradrenaline concomitant with lowered blood pressure were recently reported in long term studies with the two clonidine-like agents guanfacine and tiamenidine (Manhem and Hökfelt 1980, Hansson and Hökfelt 1981).

In contrast to the effects of clonidine on plasma noradrenaline, long term treatment with the cardioselective β-blocker metoprolol, in dosages leading to a marked reduction in blood pressure and pulse rate, did not alter the levels of plasma noradrenaline under basic conditions. Similar results were earlier reported following long term treatment with the non-selective β-blockers penbutolol and propranolol (Hansson and Hökfelt 1975, deLeeuw et al. 1977, respectively), whereas increased noradrenaline levels were found by Distler et al. (1978) and Lijnen et al. (1979) in resting patients under treatment with cardioselective β-blockers (atenolol and metoprolol) and by Rahn et al. (1978) using propranolol. Lowering of supine plasma noradrenaline was, however, reported by Brecht et al. (1976) and recently by Wilcox et al. (1981) during prolonged treatment with pindolol. Thus, measurements of plasma noradrenaline seem not to indicate that inhibition of sympathetic activity is a major reason for the antihypertensive effect of most β-blockers, pindolol being one possible exception. This rather unique property of pindolol might be related to the fact that pindolol, in addition to being a potent non-selective β-receptor blocker, possesses a rather marked intrinsic sympathomimetic activity (Gibson 1974).

Esler et al. (1981) recently showed that long term administration of propranolol reduces plasma clearance of noradrenaline by approximately 20%. This finding might indicate that the plasma levels of noradrenaline are somewhat higher in relation to sympathetic activity during medication with β-receptor blocking agents as compared to the values found without such treatment.

With respect to converting enzyme inhibition, the present studies with captopril indicate no major change of sympathetic tone under basal conditions. Similar results were previously reported (Bravo and Tarazi 1979, de Bruyn et al. 1979, Waeber et al. 1980).

As discussed above, supine plasma levels of noradrenaline have been found elevated, unaltered and even decreased following long term treatment with β-receptor blocking agents. Following physical exercise, on the other hand,

an excessive noradrenaline response has been reported by a number of authors (Hansson and Hökfelt 1975, Lütold et al. 1976, deLeeuw et al. 1977, Distler et al. 1978, Rahn et al. 1978). Again, this is in marked contrast to the pattern seen during clonidine medication and argues against the hypothesis that β-receptor blocking agents exert any major inhibitory effect on sympathetic outflow centrally or peripherally (Lewis and Haeusler 1975). Although the excessively high noradrenaline values could to some extent be due to altered metabolic clearance rate (Christensen et al. 1975, Esler et al. 1981) it seems likely that the high noradrenaline levels reflect an increased sympathetic activity in an attempt to overcome the receptor blockade introduced by the respective β-receptor blockers (Hansson and Hökfelt 1975, Planz and Planz 1981).

Even though prolonged clonidine treatment reduced PNA during all conditions studied, there was an increase both on standing and following physical exercise. This increase indicates that the sympathetic reflex mechanisms remain intact during clonidine treatment. This finding explains why an orthostatic fall in blood pressure is rarely seen during clonidine medication.

The captopril induced blood pressure reduction, mainly due to peripheral vasodilatation, could be expected to result in a compensatory increase in sympathetic activity analogous to that reported during treatment with hydralazin (Chidsey and Gottlieb 1974). Accordingly, the unaltered levels of plasma catecholamines found during treatment with captopril under resting conditions and also the unaltered noradrenaline response to physical exercise, could reflect a certain inhibitory effect of captopril on the sympathetic nervous system.

Adrenaline. Plasma adrenaline increased significantly in response to physical exercise both in male volunteers and in patients with essential hypertension before medication. This was also true following treatment with clonidine and captopril, respectively. In the metoprolol study no increase in adrenaline was seen, neither before nor during medication. This difference in adrenaline response probably was due to the higher work load applied in the studies with clonidine and captopril as compared to metoprolol. Kotchen et al. (1971) and Hartley et al. (1972) demonstrated that an increase in plasma adrenaline is seen only after heavy, prolonged exercise. The adrenaline levels following orthostatic stimulation and exercise were reduced by clonidine but not by captopril. It seems likely that this reflects an inhibitory effect of clonidine on the sympatho-adreno-medullary system. It seems likely that this is a central effect of clonidine because in studies on the effect of the postganglionic adrenergic blocking agent debrisoquine on plasma catecholamines, Flammer et al. (1979) found a diminished noradrenaline response following standing but no change in the adrenaline response.

Hansson and Hökfelt (1975) studied the effect on plasma catecholamine levels before and during prolonged penbutolol medication in hypertensive patients, subjected to the same work load as used in the present studies with metoprolol. It is of interest that exercise was followed by an increase in adrenaline during penbutolol, whereas no adrenaline response was registered during metoprolol treatment. This seems to indicate a difference in effect on adrenaline secretion by the non-selective as compared to the β_1-selective receptor blocker, probably exerted at a central level. Further support for a difference in pharmacological properties of the two compounds was seen also with respect to the effect on the adrenaline response to hypoglycemia, when penbutolol was followed by a decreased response (Hansson and Hökfelt 1976), whereas metoprolol had the opposite effect (IV).

In the present studies the plasma levels for adrenaline in supine position were very similar in healthy male volunteers (I) and the patients with essential hypertension (II, Hansson et al. 1977), the values being 0.5 ± 0.08, 0.3 ± 0.02 and 0.3 ± 0.09, respectively. Thus, our data do not as such support the view that patients with essential hypertension exhibit higher plasma adrenaline levels than normals, as maintained by Bühler (1980). On the other hand, our data do not contradict the possibility that there are two groups of hypertensive

patients, those with normal plasma levels of adrenaline and those with elevated levels (deChamplain et al. 1976).

The Renin-Angiotensin System

The present studies illustrate that the function of the renin-angiotensin system is closely related to the activity of the sympathetic system in hypertensive patients when investigated under various conditions before and during antihypertensive treatment. A close relationship between the sympathetic function and the renin-angiotensin system was originally described in normal individuals by Gordon et al. (1967). They found that stimulation of sympathetic activity by upright posture, cold or sodium deprivation was accompanied by an increase in urinary catecholamines, aldosterone and plasma renin activity, whereas a patient with autonomic insufficiency and postural hypotension showed no responses to upright posture. These findings have since been confirmed in a number of studies in animals and man. Similar results were reported also in hypertensive patients by Molzahn et al. (1972) and in the present studies a parallel increase was seen in plasma catecholamines and renin activity on assuming upright posture.

There is good experimental evidence that the simultaneously occurring changes in sympathetic activity and renin secretion, at least partly, depend on an effect via the renal sympathetic nerves and/or circulating catecholamines on the renin secreting cells. Thus, both electrical stimulation of the renal nerves and the intravenous infusion of noradrenaline was followed by increased renin secretion, as originally demonstrated by Vander (1965) in anaesthetized dogs. Both these procedures can increase renin secretion via several mechanisms. The catecholamines can exert a direct action on the renin secreting cells (Taher et al. 1976) via β-adrenergic receptors (Tobert et al. 1973). They can also constrict the afferent arterioles, which leads to an activation of the renovascular baroreceptors and also to a reduction of the delivery of sodium and chloride to the distal tubule, in turn leading to an increase in renin secretion (Reid et al. 1978).

The close correlation between sympathetic activity and renin secretion can be further demonstrated in connection with physical exercise. This was illustrated by the present results, which further extend earlier findings by other investigators. Kotchen et al. (1971) reported an increase in urinary catecholamines and plasma renin activity following graded exercise in normal individuals and similar results were presented by Chodakowska et al. (1975) and Hansson and Hökfelt (1975), who studied hypertensive patients. Hesse et al. (1978) measured the hepatic renin clearance in association with physical exercise. They came to the conclusion that changes in the hepatic elimination rate of renin do not contribute to the increase in plasma renin activity recorded during exercise. Thus, the increase in plasma renin activity following exercise most likely reflects an increase in renin secretion.

In the present study long term clonidine treatment reduced plasma renin activity both in the supine and in the upright position but there was still a significant increase in response to upright posture. These results are similar to those found in most earlier studies concerned with short term treatment with clonidine (for ref. see Keeton and Campbell 1980). So far, only few studies were published dealing with the effect of long term clonidine treatment on PRA and the results showed considerable variation (Fyhrquist et al. 1975, Karlberg et al. 1977, Weber et al. 1978). Thananopavarn et al. (1978) administered clonidine to hypertensive patients with normal plasma renin activity for 8 weeks and found a reduction of PRA. In patients with low renin hypertension the same authors found that clonidine increased plasma renin activity. In the present study, none of the patients responded to clonidine with an increase in plasma renin activity although one of them presented rather low levels of renin activity prior to medication. In the earlier studies with long term clonidine medication, no measurements of catecholamines were performed. In the present study a decrease in plasma renin activity was registered during clonidine medication together with a simultaneous fall in plasma noradrenaline, indicating a sustained inhibition of the sympathetic nervous function. In six of the eight patients, the suppression of plasma renin activity was directly correlated to the decrease in plasma noradrenaline, implying that decreased sympathetic tone at least partly was responsible for the suppression of renin secretion. Furthermore, a correlation was found between the plasma clonidine concentration and the reduction of renin activity as well as the decrease in plasma noradrenaline, which suggests that clonidine in therapeutic dosages reduces both plasma renin

activity and plasma noradrenaline in a dose dependent way. These findings support the view that clonidine reduces renin secretion by inhibition of sympathetic activity. There is some experimental evidence that clonidine can inhibit renin release also by stimulation of intrarenal α-adrenoceptors (Crayton et al. 1976, Pettinger et al. 1976, Chevillard et al. 1978), although this has been disputed (Nolan and Reid 1978).

In the present studies metoprolol significantly reduced plasma renin activity both in the supine and the upright position, indicating that β_1-receptors are engaged in the regulation of renin secretion. Similar results were earlier reported by Esler and Nestel (1973) and Bühler et al. (1975), following medication with practolol and metoprolol, respectively, but Amery et al. (1974) found no effect on plasma renin activity after the cardioselective β-blocker ICI 77082. However, in the present studies there was no significant reduction in the exercise induced increase in renin activity during metoprolol as compared to before medication. In this respect, metoprolol seems to differ from non-selective β-receptor blockers, such as penbutolol (Hansson and Hökfelt 1975) and propranolol (Bonelli et al. 1977), which very effectively lowers plasma renin activity during exercise. Taken together, these results most likely indicate that both β_1- and β_2-receptors are involved in the control of renin secretion, at least in man (Harms et al. 1978). With respect to animals, there is evidence that only β_1-receptors are involved in the control of renin secretion in most species (Kopp et al. 1981). However, there might be exceptions, because there are findings indicating that β_2-adrenoceptors are involved in regulation of renin secretion in rabbits and cats (Weber et al. 1974, Johns and Singer 1974). One possible explanation for the inability of metoprolol to significantly reduce plasma renin activity following exercise, might be the very excessive increase in plasma catecholamines occurring under these conditions (IV).

Captopril substantially increased pre-exercise plasma renin activity and simultaneously induced a significant decrease in plasma angiotensin II and plasma aldosterone. These results demonstrate that captopril is effective in inhibiting converting enzyme. The increase in plasma renin activity probably can be explained by a negative feedback of angiotensin II on plasma renin secretion. During exercise, there was a further increase in renin activity concomitant with an increase in plasma noradrenaline, suggesting

that the increase in renin activity was the result of increased sympathetic stimulation. The increase in plasma renin activity during exercise was associated with a significant increase also in plasma angiotensin II and aldosterone. This indicates that the increase in angiotensin II as measured with the RIA used, corresponded to an increase in biologically active angiotensin II. In this connection, it is of interest that despite the use of comparatively large doses of captopril, it is evident that complete inhibition of converting enzyme was not accomplished during exercise induced stimulation of the system.

The finding in the present studies that the exercise induced increase in plasma catecholamines during captopril was not altered in spite of a pronounced simultaneous decrease in plasma angiotensin II suggests that angiotensin II does not play a major role for the regulation of sympathetic activity neither under basal conditions nor during short term exercise, at least not in patients with essential hypertension. Similar results were observed by Hulthén and Hökfelt (1978) in an acute study following the administration of the converting enzyme inhibitor SQ 20.881 (teprotide) under basal conditions in hypertensive patients.

Blood Pressure and Pulse Rate

During long term clonidine medication the maximal mean arterial blood pressure reduction was correlated to the plasma level of the drug in the dosages used (200-600 µg/day). A similar relationship was previously reported in acute (Dollery et al. 1976, Frisk-Holmberg et al. 1978) and short term studies (Wing et al. 1977). Pulse rate was positively correlated to plasma noradrenaline concentration in the supine position before treatment but no correlations were registered between plasma noradrenaline and pulse rate during treatment. This indicates that the reduction of sympathetic nervous activity is not the sole mechanism responsible for the clonidine induced reduction in pulse rate under basal conditions. It seems likely that an increase in vagal tone is one factor of importance during treatment with clonidine in accordance with the finding by Walland et al. (1974) that clonidine increases vagal tone.

Metoprolol seems to be more effective than clonidine in reducing pulse rate, when the two drugs are given in dosages, which give the same reduction of blood pressure. Similar results were obtained when comparing clonidine with several other β-receptor blocking agents (Lund-Johansen 1975).

Both from theoretical and practical/therapeutic point of view it is of importance to study the effects of antihypertensive drugs not only under resting conditions but also under various kinds of stress situations, including physical exercise. The cardiovascular effects of different antihypertensive drugs might be similar during basal conditions, whereas they might differ in connection with physical exercise depending on different modes of action. Thus, Lee et al. (1979) registered a significantly reduced increase in the cardiac rate-pressure product during exercise following treatment with α-methyldopa whereas this was not the case with hydrochlorthiazide, when the two agents were given in dosages equally effective in reducing resting blood pressure. In the present study, clonidine and metoprolol reduced systolic and diastolic blood pressure as well as pulse rate both before and during submaximal work. Lund-Johansen (1974) subjected patients with essential hypertension to a stepwise increasing work load before and after 12 months of clonidine treatment. His findings were somewhat different as compared to the present study, in that clonidine did not reduce the blood pressure and pulse rate response to exercise at a near maximal work load.

With respect to the effect of captopril, blood pressure was significantly reduced during moderate exercise, whereas this was not the case at near maximal work load. This latter finding might indicate that captopril is less effective as compared to for instance clonidine in reducing blood pressure during exercise, but it should be observed that only six of the nine patients in the captopril study were able to fulfil the last three minutes of the exercise test. The finding that the absolute increase in blood pressure in response to exercise was of the same magnitude during captopril as compared to before, despite a pronounced reduction in angiotensin II formation during medication, indicates that angiotensin II is not of major importance for the regulation of blood pressure during exercise in hypertensive patients. Similar results were earlier reported with respect to healthy males following the administration of the angiotensin II antagonist saralasin under ordinary sodium intake and also following sodium depletion (Fagard et al. 1978, 1979).

Although the work load applied was somewhat lower in the study with metoprolol as compared to the studies with clonidine and captopril, the results seem to indicate that all three agents do not reduce the patients' capacity to perform submaximal work at least over a limited period of time. As treatment with clonidine and metoprolol lowers blood pressure and pulse rate during submaximal exercise, these two drugs reduce the myocardium oxygen demand without changing the working capacity. This seems not to be the case with captopril, which had no significant effect on blood pressure and pulse rate at near maximal work load. This raises the possibility that treatment with inhibitors of sympathetic function principally is more favourable for the myocardium as compared to inhibitors of converting enzyme.

D. GENERAL SUMMARY AND CONCLUSIONS

The sympathetic nervous system and the renin-angiotensin system were stimulated by standardized bicycle ergometer exercise and inhibited by antihypertensive drugs with different modes of action in order to explore the influence of the two systems on blood pressure regulation and the mode of action of the three different drugs. The studies were performed in healthy subjects and in patients with essential hypertension and included measurements of catecholamines, renin activity, angiotensin II and aldosterone in plasma.

The origin of plasma catecholamines was studied in blood samples collected simultaneously from different locations in six healthy males in the supine position before and following supine exercise. Plasma noradrenaline increased markedly in all samples, especially in the renal vein and in the coronary sinus. Calculation of the net output of noradrenaline indicates that the heart as well as the kidney contribute to the increase of noradrenaline in blood in connection with heavy exercise.

Eight patients with essential hypertension were studied under standardized conditions in the hospital before and after treatment with clonidine for 8-20 weeks with a mean dose of 506 ± 49 µg/day. Measurements were performed in the supine and the upright position and also during submaximal bicycle ergometer exercise for 20 minutes in the sitting position. Clonidine reduced blood

pressure, pulse rate, plasma noradrenaline and plasma renin activity under all conditions studied, whereas plasma adrenaline showed a decrease only in the upright position and in connection with exercise. During clonidine plasma aldosterone concentration was reduced after exercise but otherwise unaltered. The decrease in plasma noradrenaline was correlated to the decrease in diastolic blood pressure, both in the supine and in the upright position. The logarithm of plasma clonidine concentration was significantly related to the maximal percentage decrease of supine mean arterial blood pressure and to the percentage reduction of supine plasma noradrenaline concentration. In six patients, in whom clonidine caused a marked reduction of plasma renin activity, a significant positive relationship was found between the logarithm of plasma clonidine concentration and the percentage reduction of supine plasma renin activity on the one hand and between the percentage reduction of supine plasma renin activity and of supine plasma noradrenaline concentration on the other hand.

Nine patients with essential hypertension were given the cardioselective β-blocking agent metoprolol, 50-150 mg three times daily for three months. The effect of submaximal work on plasma catecholamines and renin activity was studied with the patients hospitalized under metabolic ward conditions before treatment, after one month on placebo and after three months on metoprolol. Metoprolol reduced blood pressure, pulse rate and plasma renin activity under basal conditions, but caused no changes in plasma catecholamines. During metoprolol the increase in blood pressure and pulse rate in response to submaximal work was reduced but the plasma noradrenaline response was enhanced, whereas plasma adrenaline showed no change.

Nine patients with essential hypertension were hospitalized under metabolic ward conditions and treated with captopril in gradually increasing dosages for seven days, the final dose being 600 mg/day. The patients were subjected to a graded submaximal work test for 20 minutes, in an identical manner before medication and during captopril. The blood pressure level recorded before exercise was lower during as compared to before captopril. Captopril reduced blood pressure during moderate exercise but only during seven out of ten minutes at the near maximal work load. No changes in heart rate were recorded during captopril. The plasma concentrations of angiotensin II and aldosterone were substantially reduced, whereas plasma renin activity was considerably increased

during captopril. Angiotensin II, aldosterone and renin activity increased in response to exercise both before and during captopril. The exercise stimulated increase in plasma noradrenaline and plasma adrenaline was almost identical before and during captopril.

On basis of these studies the following conclusions can be drawn:

- The kidney as well as the heart contribute to the increase in circulating noradrenaline in connection with heavy work in healthy males.

- Long term clonidine medication decreases the activity both in the sympathetic nervous system and the renin-angiotensin system. As the plasma clonidine concentration is significantly related to the maximal fall in blood pressure as well as to the reduction of supine plasma noradrenaline and plasma renin activity, it seems likely that clonidine exerts its long term antihypertensive effect via reduction of sympathetic nervous tone and as a consequence reduction of renin secretion.

- The findings that long term metoprolol treatment caused no reduction in the plasma levels of noradrenaline under basal conditions but was followed by an enhanced plasma noradrenaline response to physical exercise do not support the view that β-receptor blocking agents exert their hypotensive effect via decreased sympathetic activity. As metoprolol reduced plasma renin activity under basal conditions it seems possible that its blood pressure reducing properties at least to some extent depend on an effect on the renin angiotensin system.

- The results found under basal conditions support the view that the blood pressure lowering effect of captopril in essential hypertension is due to reduced production of angiotensin II and aldosterone rather than inhibition of sympathetic function.

- As captopril caused no change in the blood pressure response to exercise despite a pronounced reduction in angiotensin II formation, it seems likely that angiotensin II does not play a major role for blood pressure regulation during exercise.

- The fact that short term captopril treatment does not modulate the exercise stimulated increase in plasma noradrenaline and plasma adrenaline, in spite of a pronounced simultaneous decrease in angiotensin II, indicates that angiotensin II plays no major role for the regulation of the sympathetic nervous activity in patients with essential hypertension.

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Plasma catecholamine levels in the coronary sinus, the left renal vein and peripheral vessels in healthy males at rest and during exercise

By

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Abstract

Manhem, P., H. Lecerof and B. Hökfelt. *Plasma catecholamine levels in the coronary sinus, the left renal vein and peripheral vessels in healthy males at rest and during exercise.* Acta physiol. scand. 1978. *104*. 364–369.

Noradrenaline and adrenaline were determined in blood samples from the brachial vein, the brachial artery, the left renal vein and the femoral vein in 6 healthy males (aged 23–35 y). In 3 of the subjects catecholamines were determined also in blood from the coronary sinus. All samples were taken simultaneously in supine position after 30 min of rest and then in connection with exercise in supine position using a bicycle ergometer, firstly with a work load of 50 W for 9 min and secondly with a work load of 150 W for the same period of time. Under resting conditions the catecholamine levels were about the same at all locations, the mean for noradrenaline being 1.59 nmol/l with a range of 1.30–2.11 nmol/l and for adrenaline 0.46 nmol/l and 0.23–0.65 nmol/l, respectively. At 50 W the noradrenaline concentration increased significantly in the brachial artery, the left renal vein and the femoral vein, whereas adrenaline increased significantly only in the femoral vein. At 150 W the noradrenaline content increased markedly in all samples, especially in the left renal vein (mean increase 13.02 nmol/l) and the coronary sinus (mean increase 13.06 nmol/l). Adrenaline concentration increased significantly in the brachial artery and the femoral vein. At 150 W the mean net output of noradrenaline as estimated from the calculated flows and actual AV-differences amounted to 2.25 nmol/min from the heart and to 0.36 nmol/min from the kidney.

Key words: Plasma catecholamines, renal, cardiac, exercise

In blood from healthy, resting individuals the ratio of noradrenaline to adrenaline is about 5:1 but both absolute and relative amounts vary widely (Engelman and Portnoy 1970). Circulating noradrenaline is believed to originate mainly from the sympathetic nerve endings, adrenaline from the adrenal medulla. The concentration of both these catecholamines in blood increases during exercise (Kotchen *et al.* 1971, Christensen and Brandsborg 1973, Chodakowska *et al.* 1975 and Hansson and Hökfelt 1975). So far, only few studies have been performed with the aim of defining the more precise sources of the circulating catecholamines. We have determined the catecholamine concentration in blood at different levels in the arterial and venous vascular system of healthy men at rest and after standardized, light and heavy physical work.

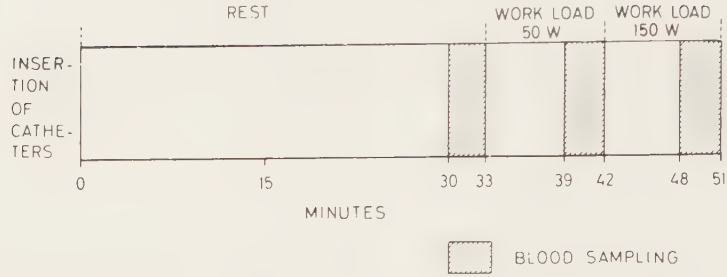

Fig. 1. Experimental design.

Material and methods

Studies were performed in 6 healthy, physically well-trained volunteers with a mean age of 25.8 years (range 23–35). The investigations were carried out on an ambulatory basis. The subjects were instructed not to eat or smoke after 10 o'clock p.m. on the day prior to the investigations, which in all cases took place at 8–11 a.m.

Consent was given by each subject after complete description of the protocol. The study was approved by the Ethical Committee of the Medical Faculty at the Lund University.

Polyethylene catheters PE 160 were placed by means of the Seldinger technique in the brachial artery, the brachial vein and the femoral vein and a Gensini catheter 7 F in the left renal vein. In 3 of the subjects a Goodale-Lubin catheter 7 F was inserted into the coronary sinus through a cubital vein. The position of each catheter was controlled by X-ray. After the insertion of the catheters, the subjects rested in supine position for at least 30 min before blood samples were taken for the determination of the basal catecholamine concentrations.

Physical work consisted of bicycling in supine position for 9 min with a work load of 50 W and immediately afterwards another 9 min with a load of 150 W (Fig. 1). The bicycle used was an electrically braked variable load ergometer (Siemens-Elema). The ECG was recorded continuously during the work test.

In two of the three subjects, in which catheterization of the coronary sinus was undertaken, the maximum oxygen uptake ($\dot{V}O_2$ max) was determined. These studies showed that a work load of 150 W represents 75 per cent of the maximum working capacity. At a work load of 150 W the mean heart rate for all participants was 165 beats/minute. Thus, it is evident that a work load of 150 W represented relatively hard work for the group as a whole.

Blood samples for the determination of catecholamines were drawn simultaneously from all catheters at rest, immediately prior to work and then during the last 3 min of work at 50 and 150 W, respectively (Fig. 1).

Plasma noradrenaline and adrenaline were analysed using a double labelling isotope derivative technique (Engelman and Portnoy 1970). In order to exclude that the blood drawn from the left renal vein was contaminated with adrenal vein blood, the concentration of cortisol was determined in the left renal vein and compared to the cortisol concentration at other levels. Cortisol was estimated using fluorimetry (Clark and Rubin 1969).

When applying a work load comparable with the heaviest used in the present studies, Lange-Anderson (1968) found an increase in the coronary blood flow from about 250 ml/min at rest to about 1 000 ml/min during work. Simultaneously, the kidney blood flow decreased from about 1 100 ml/min to about 300 ml/min. Using these figures and the formula:

$$\text{AV-difference} \quad \text{plasma catecholamine concentration} \times \text{plasma flow},$$

we have calculated the net output of catecholamines from the coronary sinus and from the kidney in connection with exercise at the work load of 150 W.

Statistical analysis was performed by Student's t-test for paired observations. The values are reported as mean $\pm$ SE and the level of significance taken as $p < 0.05$.

TABLE I. Noradrenaline, adrenaline and cortisol in blood samples taken from the brachial artery, the brachial vein, the left renal vein, the femoral vein and the coronary sinus at rest, respectively at 50 W and 150 W work load.

	Noradrenaline nmol l						Adrenaline nmol/l						Cortisol nmol/l					
	0		50 W		150 W		0		50 W		150 W		0		50 W		150 W	
	mean ± SE		mean ± SE		mean ± SE		mean ± SE		mean ± SE		mean ± SE		mean ± SE		mean ± SE		mean ± SE	
A. brachialis n = 6	1.33	0.22	2.15*	0.41	9.54*	2.39	0.65	0.45	0.74	0.13	2.17*	0.60	260.0	23.6	223.0*	23.2	187.5***	26.8
V. brachialis n = 6	2.11	0.54	2.37	0.53	7.87**	1.66	0.50	0.08	0.50	0.09	1.77	0.63	279.9	27.7	260.2	30.8	211.0**	28.8
V. renalis sin n = 6	1.75	0.41	2.92*	0.45	14.77*	4.83	0.41	0.03	0.49	0.05	2.56	1.78	208.0	20.6	166.6	25.4	164.5*	26.2
V. femoralis n = 6	1.30	0.23	2.08*	0.39	9.38*	2.46	0.23	0.02	0.53**	0.08	1.79*	0.55	256.5	27.1	222.6***	24.6	200.3*	26.5
S. coronarius n = 3	1.44	0.16	3.06	0.32	14.5	4.65	0.49	0.06	0.67	0.09	1.86	0.39	227.6	42.5	218.3	44.8	185.0**	42.9

* Denotes significant alterations compared to basal value at the level of significance, where $p < 0.05$.
** where $p < 0.01$ and
*** where $p < 0.001$.

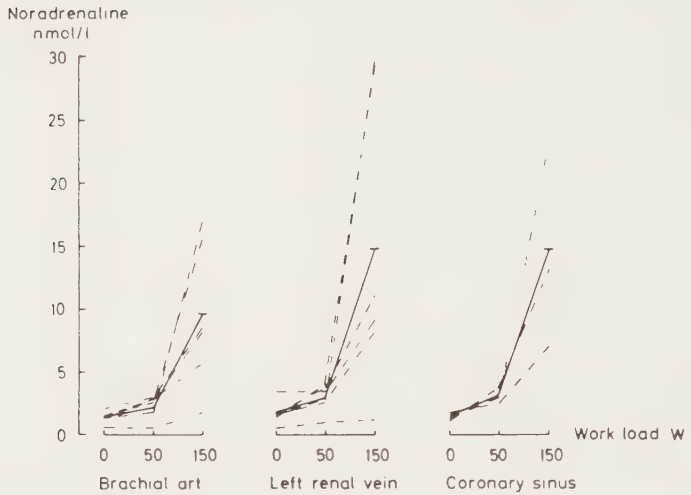

Fig. 2. Noradrenaline in blood taken from the brachial artery, the left renal vein and the coronary sinus at rest, and at 50 W and 150 W work load respectively. Broken lines indicate the values from the individual subjects, solid line indicates the mean values.

Results

The plasma cortisol concentration decreased stepwise at all sites following work and the level in the renal vein did not exceed that found in other locations at the respective times. Thus, there was no significant contamination of blood from the adrenal vein (Table I).

At rest the concentration of noradrenaline was similar in blood samples from all locations and increased uniformly at 50 W (Table I). The increase was statistically significant in samples from the brachial artery, the left renal vein and the femoral vein. At 150 W the noradrenaline content increased considerably at all sample sites. However, there was a greater increase of the noradrenaline concentration in blood from the left renal vein ($n = 6$) and from the coronary sinus ($n = 3$) as compared with other locations. Thus, concentration of noradrenaline in the left renal vein significantly exceeded the concentrations in the brachial vein, brachial artery and femoral vein (Fig. 2). In the coronary sinus the noradrenaline concentration at the heavier work load increased to the same level as in the left renal vein, but the increase was not statistically significant, possibly because of the relatively few subjects studied (Fig. 2).

The adrenaline concentration in samples taken at rest was of the same magnitude at all sites and increased in all locations except the brachial vein following a work load of 50 W (Table I) and at all sites following 150 W.

At 150 W the net output of noradrenaline, calculated as previously mentioned, amounted to 2.25 nmol/minute from the heart and 0.36 nmol/minute from the kidney. Similar calculations revealed no significant addition of noradrenaline from the heart and the kidney under basal conditions and at a work load of 50 W. No other significant AV-differences were registered.

Discussion

Yamaguschi (1975) stimulated the sympathetic nerves to the heart in the dog and concomitantly measured the catecholamines in the blood from the coronary sinus. He concluded that measurements of noradrenaline in blood from the coronary sinus can be used as an indication of the amount of sympathetic neurotransmittor released in heart tissue. Our results seem to support his idea.

Our findings indicate that the heart as well as the kidneys contribute to the increase of noradrenaline in blood occurring in connection with heavy work. It seems likely that the release of noradrenaline in the heart during physical work is even greater than estimated from our figures since part of the noradrenaline released is subject to re-uptake in the neuron, part is taken up by extra neuronal tissues and some is metabolized (Kopin 1977). Moreover, it seems probable that some of the noradrenaline transported to the heart muscle from other locations is taken up by heart tissue (Ginn and Vane 1968).

Miura *et al.* (1976) measured the concentrations of adrenaline and noradrenaline in plasma taken from different parts of the circulatory system in 22 patients with heart disease, at rest and following handgrip exercise. According to their calculations the contribution of noradrenaline from the coronary sinus was rather small not only at rest but also during exercise. This difference in their results and ours probably is due to the low work load applied in the experiments of Miura *et al.*

Previously, no attempts seem to have been made to calculate the noradrenaline output from the kidney during heavy work. Our results probably reflect considerably increased renal sympathetic activity at heavy work.

In healthy, resting individuals Vendsalu (1960) found that the adrenaline concentration was significantly higher in the brachial artery than in the brachial vein, while the opposite was true for noradrenaline. In our study we found no such differences, either at rest or in connection with work. We can offer no definite explanation for the discrepancy between Vendsalu's findings and ours. However, it may be pointed out that he investigated a larger number of individuals than we did and that the differences found by Vendsalu, although statistically significant, were rather small. Furthermore, Vendsalu used a fluorometric method to measure catecholamines, which is less capable of differentiating between noradrenaline and adrenaline as compared to the method used by us.

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Prolonged Clonidine Treatment: Catecholamines, Renin Activity and Aldosterone Following Exercise in Hypertensives

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ABSTRACT. Eight patients with essential hypertension, WHO grade I–III, were studied under standardized conditions in a metabolic ward, before and after 8–20 weeks of treatment with clonidine in a maintenance dose of 300–600 μg/24 h. Before clonidine, plasma noradrenaline concentration (PNA), plasma adrenaline concentration (PA), plasma renin activity (PRA) and plasma aldosterone concentration (PAC) increased in response to standing and submaximal exercise for 20 min. PNA was positively correlated to pulse rate in the supine position ($R=0.74$, $p<0.05$) and the increase in PNA to the increase in pulse rate during exercise (min 10, $R=0.73$, $p<0.05$; min 15, $R=0.79$, $p<0.05$; min 20, $R=0.74$, $p<0.05$). No other significant correlations were found between PNA, PA, PRA and PAC on the one hand and blood pressure (BP) and pulse rate on the other. Clonidine reduced BP, pulse rate, PNA and PRA under all conditions studied. PA was reduced in the upright position and in connection with exercise. PAC was reduced during clonidine after exercise but otherwise unaltered. The clonidine-induced decrease in PNA was positively correlated to the decrease in diastolic BP both in the supine ($R=0.76$, $p<0.05$) and in the upright ($R=0.80$, $p<0.05$) position. Thus, long-term clonidine treatment lowered the BP and pulse rate, at least partly by reducing sympathetic activity via a central mechanism. However, clonidine did not block the sympathetic reflex mechanisms engaged in the maintenance of BP in the upright position. During clonidine, the adrenaline values were lower than before treatment in the supine and in the upright position and also following exercise, indicating that clonidine exerts an inhibitory effect on the sympatho-adreno-medullary system.

Key words: aldosterone, catecholamines, clonidine, exercise test, hypertension, renin.

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The α-receptor agonist clonidine (Catapresan®) acts centrally to decrease sympathetic tone (21, 31, 32) and to increase vagal activity (20, 31). It effectively reduces blood pressure (BP) in animals (16) and in man (6, 17). Given acutely, clonidine is followed by a decrease in urinary catecholamines, plasma renin activity (PRA) and urinary aldosterone in patients with essential and renovascular hypertension (2, 3, 17). With respect to prolonged treatment, earlier studies have demonstrated an initial and transitory reduction of PRA (11, 19). However, reduced values for renin activity and catecholamines in plasma were recently reported also following medication with clonidine for 4–6 weeks (15).

Results are presented here concerning the effect of treatment with clonidine for 8–20 weeks on BP and pulse rate in relation to catecholamines, renin activity and aldosterone in plasma. The studies were performed in 8 patients with essential hypertension under basal conditions and following physical exercise. Studies concerning the long-term effects of clonidine on sympathetic activity and the renin–angiotensin–aldosterone system are of principal importance not only to explore the mode of action of the drug as such, but also to try and gain information on the mechanisms engaged in the pathogenesis of various types of hypertension.

PATIENTS

Seven males and one female with untreated hypertension were studied. BP was measured by sphygmomanometry, diastolic levels being taken at Korotkoff phase 4. All patients had an ambulatory BP of 160/105 mmHg or higher, measured on two occasions after at least 20 min in recum-

Parts of these studies were presented at an international symposium on Central Adrenaline Neurons: Basic Aspects and Their Role in Cardiovascular Functions, Wenner-Gren Center International Symposium Series, vol. 33, Pergamon Press, 1980.

Abbreviations: BP=blood pressure, PNA=plasma noradrenaline concentration, PA=plasma drenaline concentration, PRA=plasma renin activity, PAC=plasma aldosterone concentration, CV=coefficient of variation.

Table I. *Clinical and laboratory findings in 8 patients with hypertension*

KW=Keith-Wagener grade, LVH=left ventricular hypertrophy

Pat no.	Age (y.)	Sex	B. wt. (kg)	Height (cm)	Ambulatory BP (mmHg)	Eye ground changes (KW)	Cardiac size (ml/m²)	ECG	Supine PRA (μg/l/3 h)	U-aldosterone (nmol/24 h)	U-noradrenaline (nmol/24 h)
I	37	♂	56	172	160/105	I	430	LVH	5.9	24	168
II	52	♂	98	178	160/105	I	480	LVH	1.3	29	114
III	48	♂	96	165	175/110	I–II	370	Normal	1.2	49	123
IV	33	♂	93	176	190/120	I	530	LVH	5.7	80	161
V	46	♂	73	172	205/125	I	360	Normal	6.3	31	143
VI	49	♀	50	163	180/110	I	250	LVH	4.6	70	170
VII	55	♂	77	171	170/115	II	470	Normal	0.9	33	94
VIII	36	♂	99	192	200/120	II	290	Normal	2.4	36	143
Normal range									0.5–2.0	14–40	60–300

bent position. All were regarded as having essential hypertension, seven WHO grade I–II, one grade III (Table I). Patients I, IV, V and VI had relatively high supine PRA and patients IV and VI rather high urinary aldosterone excretion. Two patients had atoxic nodular goiter and were treated with l-thyroxine, 0.10–0.15 mg/day, before and throughout the experimental period. No evidence of additional disease was found and no medication except clonidine was given. The female patient was studied during the same menstrual phase before and during clonidine. The investigations were approved by the Ethical Committee of the Medical Faculty, University of Lund. Informed consent was obtained from all patients.

METHODS

Procedures

The patients were hospitalized in a metabolic ward for a period of 10 days before treatment and for another 10 days 8–20 weeks after initiation of clonidine treatment. They were maintained on a constant diet containing 135 mmol Na and 95 mmol K per day. During the two hospital periods the patients were subjected to identical investigations.

On days 3 and 4, referred to below as "basal days", blood samples were collected in EDTA tubes at 8 a.m. with the patients fasting and at bed rest since 11 p.m. the day before. Another series of blood samples were collected at 12.00 on the same day in upright position after 30 min of walking. Urinary sodium excretion was determined in 24-hour urine specimens collected on basal days.

All patients were subjected to a standardized submaximal work test on day 10, at 9 a.m. after an overnight fast. The test consisted of exercise for 20 min with the patients seated on a bicycle ergometer. The work load was designed to give a steady state pulse rate corresponding to about 80% of maximal working capacity according to the nomogram of Åstrand (1). The work load was determined in advance for each patient in the medication-free state. Before and every 5 min during exercise, blood was drawn from an antecubital vein through an indwelling catheter kept patent by a slow saline infusion.

All plasma samples were separated and stored at –20°C until assayed for catecholamines, renin activity and aldosterone. All samples collected from each patient under the respective conditions before and during clonidine were analyzed in one and the same series.

On basal days, BP and pulse rate were measured as follows: In the supine position after overnight bed rest at 8 a.m., 8.30 a.m., 9 a.m. and 9.30 a.m. and after 30 min bed rest at 1 p.m., 3 p.m. and 7 p.m. After 2 min in the upright position at 1 p.m., 3 p.m. and 7 p.m. In connection with submaximal work, BP and pulse rate were recorded immediately before the start of the test and then every 5 min during exercise. On all occasions, BP and pulse rate were measured by a specially trained nurse.

Plasma noradrenaline (PNA) and adrenaline (PA) concentrations were determined by a double isotope derivative technique (9). For noradrenaline the intra- and interassay coefficient of variation (CV) was 2.7% and 3.7% (at PNA mean 2.5, range 1.28–3.70 nmol/l), respectively, and for adrenaline 6.2% and 9.7% (at PA mean 0.46, range 0.25–0.73 nmol/l), respectively. PRA was determined as the amount of angiotensin I generated during 3 hours' incubation at pH 6.0 (4, 12). The intra- and interassay CV was 7.7% and 8.3% (at PRA mean 3.9, range 1.21–11.80 μg/l/3 h), respectively. Plasma aldosterone was extracted on a Sephadex LH 20 column and the concentration in plasma (PAC) was determined by a radioimmunoassay as described by Ito et al. (18). The intra- and interassay CV was 11.9% and 12.1% (at PAC mean 0.48, range 0.25–0.78 nmol/l), respectively. Sodium and potassium in blood and urine were measured by standard laboratory techniques.

Medication

Clonidine was administered orally in an initial dose of 50 μg four times daily (at 7 a.m., 1, 7 and 11 p.m.). All

U-adrenaline (nmol/24 h)	Rapid sequence i.v. pyelogram	Isotope renogram	Renal angiography
66	Normal	Normal	–
20	Normal	Delayed uptake right kidney	Normal
16	Normal	Normal	–
52	Normal	Normal	–
18	Slight parenchymal reduction pars inferior left kidney	Normal	Nephrosclerosis
24	Normal	Normal	–
31	Normal	Normal	–
20	Normal	Normal	–
0–60			

patients were seen regularly at intervals of 1–4 weeks. If BP had not fallen to 150/95 or less, the dose was increased until satisfactory BP control was obtained. The final dosage—600 μg/day in five patients, 450 in one and 300 in two—was maintained for 4–16 weeks on an ambulatory basis and then continued during the second hospitalization.

Statistical evaluation

Statistical analysis was performed by non-parametric methods: Data presented are given as mean ± S.E. Statistical significance of differences was assessed by Wilcoxon rank sum test and Spearman's correlation coefficient (R) was used. The level of significance was taken as $p<0.05$.

RESULTS

Basal conditions

Systolic and diastolic BP did not change significantly in response to upright position before medication, but pulse rate increased ($p<0.01$). Clonidine treatment reduced systolic and diastolic BP as well as pulse rate in supine and erect position (Fig. 1). On clonidine, pulse rate increased in response to upright position ($p<0.01$) whereas BP showed no change.

PNA increased in response to standing before clonidine (Fig. 2). Clonidine reduced supine and upright PNA, but PNA still increased in response to standing, although the levels were lower during medication.

PA increased in the standing position before and during clonidine (Fig. 2). Clonidine caused no change in supine PA but the level in response to standing was lower as before medication.

PRA increased in response to standing before and during clonidine (Fig. 3). Clonidine reduced the levels of PRA in the supine and upright position.

PAC increased in response to standing (Fig. 3). Clonidine had no effect on PAC either in the supine or in the upright position.

Correlations were estimated for PNA, PA, PRA and PAC in relation to supine and upright BP and pulse rate before and during clonidine treatment. Both absolute values and absolute and percentage differences were calculated. PNA was positively correlated to pulse rate ($R=0.74$, $p<0.05$) in supine position before treatment. The percentage decrease in PNA was positively correlated ($R=0.76$, $p<0.05$) to the decrease in diastolic BP in supine position. The absolute decrease in PNA was correlated to the decrease in diastolic BP ($R=0.80$, $p<0.05$) in erect position. No other correlations were significant.

The decrease in PRA during clonidine treatment was not correlated either to the decrease in PNA or to systolic and diastolic BP and pulse rate in supine and erect position. All PRA values in the

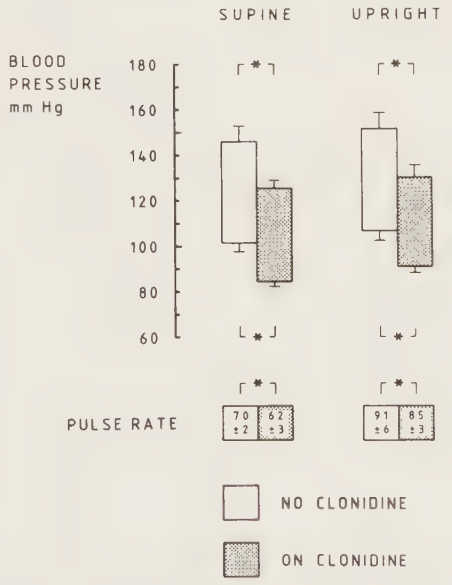

Fig. 1. BP and pulse rate in supine and upright position before and during clonidine treatment for 8–20 weeks (mean ± S.E.). *$p<0.05$.

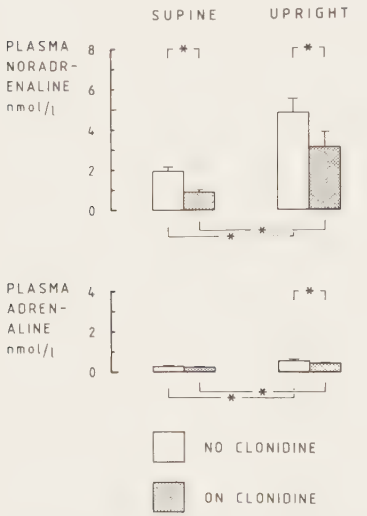

Fig. 2. PNA and PA in supine and upright position before and during clonidine treatment for 8–20 weeks (mean ±S.E.). $^{*}p<0.05$.

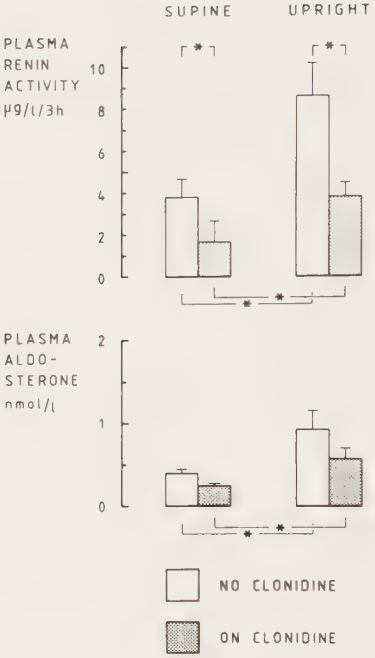

Fig. 3. PRA and PAC in supine and upright position before and during clonidine treatment for 8–20 weeks (mean ±S.E.). $^{*}p<0.05$.

supine as well as upright position were positively correlated to the same PAC values both before ($R=0.79$, $p<0.05$) and during clonidine treatment ($R=0.59$, $p<0.05$).

Serum potassium was 4.0±0.1 mmol/l before (on day 3) and 4.1±0.1 during clonidine treatment ($p>0.05$). Serum sodium was 141.8±0.8 mmol/l before and 140.6±0.6 during clonidine treatment ($p>0.05$). Urinary sodium was 112.8±10.1 mmol/24 h on day 3 and 92.5±10.0 on day 4 before treatment and 138.4±7.5 and 105.6+7.4 during treatment ($p>0.05$ in both instances). The mean body weight was 80.3±7.3 kg before (on day 3) and 80.3±7.1 during clonidine treatment ($p>0.05$).

Submaximal work

Systolic and diastolic BP and pulse rate were reduced by clonidine before and during submaximal work (Fig. 4).

PNA increased in response to exercise before and during clonidine treatment (Fig. 5), but all levels were lower than before clonidine. A similar pattern was seen for PA (Fig. 5).

PRA increased after 20 min of bicycling before and during clonidine administration, but the absolute levels were reduced by clonidine (Fig. 6). After 20 min of work, PAC increased both before and following clonidine (Fig. 6). During medication the absolute PAC level tended to be lower before work ($p=0.07$) and was clearly reduced after exercise as compared to the values recorded without medication.

Correlations were estimated for PNA, PA, PRA and PAC in relation to BP and pulse rate. The increase in PNA in response to exercise was positively correlated to the increase in pulse rate before medication (min 10, $R=0.73$, $p<0.05$; min 15, $R=0.79$, $p<0.05$; min 20, $R=0.74$, $p<0.05$). No other correlations were significant.

Pre- and postexercise PRA was positively correlated to pre- and postexercise PAC ($R=0.83$, $p<0.01$) before clonidine, the correlation being close to significant ($R=0.45$, $0.1>p>0.05$) during clonidine.

DISCUSSION

The purpose of this study was to investigate the effect of long-term treatment with therapeutically

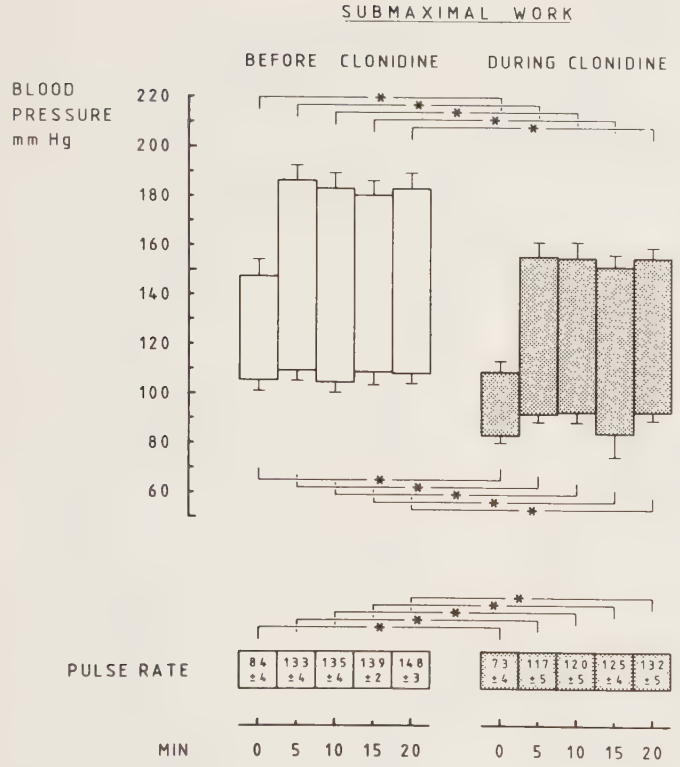

Fig. 4. BP and pulse rate in connection with submaximal exercise before and during clonidine treatment for 8–20 weeks(mean±S.E.)*$p<0.05$.

active dosages of clonidine on the plasma levels of catecholamines, renin activity and aldosterone under basal conditions and in connection with exercise. All samples for the different analyses were drawn under standardized conditions. A diet constant in sodium and potassium content is of importance, because sodium intake has been shown to influence the plasma levels of noradrenaline (30) as well as renin activity and aldosterone (28, 33). Clonidine treatment reduced the plasma levels of noradrenaline and renin activity in all conditions studied, the levels of adrenaline in most instances and the levels of aldosterone occasionally. It is of importance that earlier studies with a design similar to ours showed that placebo treatment causes no change in the plasma levels of catecholamines and renin activity (13).

It seems likely that the changes in plasma noradrenaline content recorded under various conditions before and during treatment with clonidine reflect changes in sympathetic nervous activity. This assumption is supported by the finding of increased levels of PNA following sympathetic activation in connection with standing and physical exercise, which is in agreement with earlier findings (8, 13, 14). Subnormal levels of noradrenaline are seen in tetraplegic subjects with chronic cervical spinal cord transection (26) and following ganglionic blockade (23). The interpretation that reduced plasma noradrenaline levels during medication with clonidine reflect reduced sympathetic activity is supported by experimental studies in which clonidine was shown to reduce sympathetic activity via a central mechanism (21).

The hypotensive effect of clonidine has been related to its sedative effect (5, 7). On the basis of acute studies, Wing et al. (34) discussed the possibility that the reduction of plasma noradrenaline

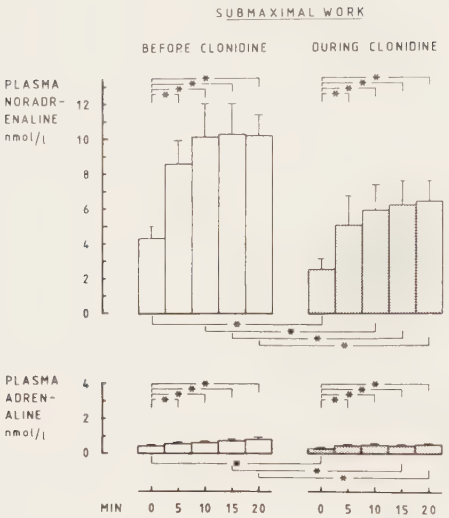

Fig. 5. PNA and PA in connection with submaximal exercise before and during clonidine treatment for 8–20 weeks (mean±S.E.). $*p<0.05$.

could be due to the sedation as such. Although our patients experienced slight sedation during the initial part of the treatment, this was not the case after 8–20 weeks. Accordingly, sedation seems not to play a major role for the decrease in plasma noradrenaline during clonidine, at least not during prolonged medication.

Plasma noradrenaline increased considerably in upright position and following physical exercise also during clonidine, indicating that the sympathetic reflex mechanisms remain intact during prolonged treatment with clonidine. This probably contributes to counteract an orthostatic fall in BP in spite of the inhibition of sympathetic activity induced by clonidine.

Both standing and physical exercise probably activated the adrenal medulla to secrete increased amounts of adrenaline, as evidenced by elevated plasma levels. During clonidine, the adrenaline response was less pronounced, most likely reflecting an inhibition by clonidine of the sympatho-adrenomedullary system. It seems likely that this effect of clonidine was exerted centrally because in studies of the effect of the postganglionic agent debrisoquine on plasma catecholamines, Flammer et al. (10) found a diminished plasma noradrenaline response following standing but no inhibitory effect on the adrenaline response.

As mentioned in the introduction, previous studies concerning the long-term effect of clonidine on PRA have given varying results. Fyhrquist et al. (11) studied 15 patients with essential hypertension, WHO grade I–II. During medication with clonidine, 75 μg 3 times daily, a reduction of PRA was seen initially in some patients, but after three months of treatment the levels were back to or even higher than pretreatment levels. Clonidine treatment resulted in a significant reduction of BP over the period studied. As compared to our studies, the clonidine dosages given were smaller and all measurements were performed on an ambulatory basis. In another ambulatory study, Karlberg et al. (19) treated 18 patients with essential hypertension, WHO grade I–II, with clonidine, 225 μg/day, for two weeks and recorded a decrease in BP and PRA. When gradually increasing the dosages to as high as 1 150 μg/day, no statistically significant changes in PRA were recorded. Neither Fyhrquist et al. (11) nor Karlberg et al. (19) measured plasma catecholamines in their patients.

Renin secretion is regulated via several mechanisms, such as renal artery mean BP, sympathetic activity/circulating catecholamines and sodium load at the level of macula densa (28). In our studies, no correlation was found between the plasma levels of catecholamines and PRA under any of the conditions studied before and during clonidine, except following exercise before clonidine. Thus, in spite of the simultaneous fall in catecholamines and PRA during clonidine medication, no statistical correlation between them could be established, with only one exception. The body weight of our patients remained unchanged during clonidine treatment, probably excluding fluid retention as a major determinant for the PRA decrease. Furthermore, no change in serum sodium concentration or urinary sodium excretion was observed during treatment. This might imply that fluid redistribution resulting in an increased circulatory volume was not of major importance for the decrease in PRA during clonidine.

On the basis of studies in rats, Pettinger et al. (29) proposed that clonidine could exert a direct inhibitory effect on renin secretion via a stimulation of α-receptors in the kidney. No such effects were, however, seen in studies in dogs, when renal perfusion pressure was controlled by means of an

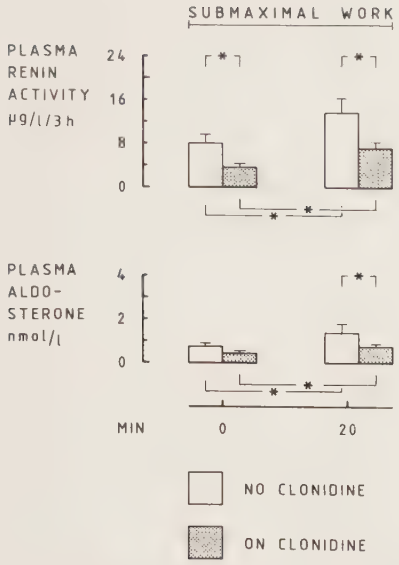

Fig. 6. PRA and PAC before and after submaximal exercise before and during clonidine treatment for 8–20 weeks (mean±S.E.) $*p<0.05$.

aortic clamp (27). Also, no effect of clonidine on PRA was recorded in recently performed studies in tetraplegic patients (25).

In our studies, plasma aldosterone levels were significantly correlated to the levels of PRA under all conditions studied. Significant decrease in plasma aldosterone was found also by Karlberg et al. (19) following treatment with clonidine in a dose of 225 μg per day, parallel with a decrease in PRA. Again, further treatment with clonidine in higher dosages was not connected with a reduction of the plasma aldosterone levels (19).

So far, only a few studies have been published concerning the effect of clonidine on working capacity. Lund-Johansen (24) subjected patients with essential hypertension to a stepwise increasing work load before and after 12 months of clonidine treatment. He found that clonidine treatment reduced the BP and pulse rate responses following exercise, except when applying a near maximal work load. Our studies showed that patients retain their ability to perform submaximal work also after prolonged treatment with clonidine. Furthermore, the BP and pulse rate responses as well as plasma catecholamine and PRA responses were less pronounced during than before clonidine treatment when the same work load was applied. Similar results with respect to BP and pulse rate were reported by Koch (22) in an acute study.

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PLASMA CLONIDINE LEVELS IN RELATION TO BLOOD PRESSURE, CATECHOLAMINES AND RENIN ACTIVITY DURING LONG TERM TREATMENT OF HYPERTENSIVE PATIENTS

By

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ABSTRACT

Eight patients with essential hypertension were treated with oral clonidine 50 μg four times daily for four weeks followed by 75-150 μg four times daily for another 4-16 weeks. Before treatment and at the end of each treatment period the patients were hospitalized and plasma clonidine concentration (P-CLON) determined repeatedly during one dosage interval. The relation of P-CLON to blood pressure, heart rate, plasma noradrenaline (PNA) and plasma renin activity (PRA) as well as conventional kinetic parameters were calculated. With the two dosages used the half life of clonidine absorption was 1.05 and 0.83 hours, the biological half life 8.7 and 7.9 hours and total oral clearance 4.7 and 5.0 ml · min^{-1} · kg^{-1}, respectively. The logarithm of P-CLON was significantly related to the maximal percentage decrease of mean arterial blood pressure (MAP) ($r = 0.91$, $p < 0.001$) in five patients, whereas three patients showed no further decrease of MAP with an increase of plasma clonidine levels. The logarithm of P-CLON was significantly related to the percentage reduction of PNA ($r = 0.67$, $p < 0.01$). In six patients, in whom clonidine caused a marked reduction of PRA, a significant relationship was found between the logarithm of P-CLON and the percentage reduction of PRA ($r = 0.91$, $p < 0.001$) and also between the percentage reduction of PRA and PNA ($r = 0.74$, $p < 0.01$).

INTRODUCTION

In acute experiments in animals clonidine lowers blood pressure by decreasing sympathetic tone[12, 14, 15] and increasing vagal activity[11, 14]. In patients with essential hypertension it induces a fall in plasma noradrenaline (PNA) and plasma renin activity (PRA) concomitant with a reduction of blood pressure[10]. Due to the methodological difficulties involved in the measurement of the low therapeutic plasma clonidine concentrations (P-CLON) only limited knowledge exists concerning the relationship between P-CLON and its effects in man[5, 8, 19]. In the present study we investigated the relationship of plasma clonidine levels to blood pressure, plasma catecholamines and plasma renin activity in eight patients with essential hypertension treated with clonidine for altogether 8-20 weeks.

METHODS

Seven males and one female with untreated hypertension were studied. Blood pressure was measured by sphygmomanometry, diastolic levels registered at Korotkoff phase 4. All patients had an ambulatory blood pressure of 160/105 mmHg or higher as measured on two occasions after at least 20 min in recumbent position. All were regarded as having essential hypertension, seven with WHO grade I-II, one WHO grade III. Two patients had atoxic nodular goiter and were treated with l-thyroxine 0.10-0.15 mg/day. No evidence of additional disease was found and no medication except clonidine was given. In the female patient the studies were performed during the same menstrual phase at each hospitalization period. Further details regarding these patients were published in a previous paper (13). The investigations were approved by the Ethical Committee of the Medical Faculty, University of Lund. Informed consent was obtained from all patients.

Clonidine (Catapres^{R}) was given orally in divided doses (7 a.m., 1, 7 and 11 p.m.), starting with 200 µg daily for 4 weeks. Thereafter, the dose was increased stepwise until the ambulatory blood pressure had been reduced to or below 150/95 mmHg. The final dosage amounted to 600 µg/day in five patients, 450 µg in one and 300 µg in two.

The patients were hospitalized for specialized studies (1) before treatment, (2) after four weeks on 200 µg clonidine daily (= 0.67 ± 0.08 µg/kg BW), and (3) after another 4-16 weeks on 300-600 µg daily (mean 506 ± 49 µg = 1.64 ± 0.09 µg/kg BW). For technical reasons one patient was not investigated when on the lower clonidine dosage. Blood samples were drawn in EDTA tubes at 8 a.m. with the patients fasting, non-smoking and at bed rest since 11 p.m. the previous day. The plasma was immediately separated by centrifugation at +4°C and stored at -20°C until assayed for catecholamines and renin activity. Ice-chilled tubes containing 20 mg ascorbic acid were used for the later determination of catecholamines. For each patient the samples collected under the respective conditions were analyzed in one and the same assay run. On the same day blood was collected in heparinized tubes for the determination of clonidine concentration, samples being drawn at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 hours after the administration of the drug at 7 a.m. Plasma was separated and stored at -20°C until assayed. Blood pressure and pulse rate were registered in the supine position after a minimum of 30 min rest at 1.0, 1.5, 2.0, 2.5 and 6.0 hours following clonidine administration.

Plasma noradrenaline and adrenaline were determined by a double isotope derivative technique[7]. For noradrenaline the intra- and inter-assay coefficient of variation (CV) was 2.8% (when measured at a range of 0.4-3.8 nmol/l) and 3.8% (range 0.4-3.8 nmol/l), respectively, and for adrenaline 6.3% (range 0.17-0.77 nmol/l) and 9.4% (range 0.17-0.77 nmol/l), respectively. Plasma renin activity was determined as the amount of angiotensin I generated during 3 hours incubation at pH 6.0[1, 9]. The intra- and inter-assay CV was 7.7% and 8.3%, respectively. Clonidine was analyzed by glass capillary gas chromatography according to Edlund[6]. The sensitivity was 12 pmol/l and the recovery using this method was 40-60% for concentrations in the range of 0.4-4.4 nmol/l.

Analyses of data

The plasma clonidine concentration-time data were fitted to the biexponential function (equation 1) by the nonlinear least-square regression program NONLIN run on an IBM 360 computer. In the fitting procedure a special subroutine of the NONLIN-program was used allowing computation of unequal and repetitive doses and dosing intervals. The data points were weighted according to the reciprocal of their standard deviation (1/S.D.). The goodness of fit of observed

data was based on coefficient of correlation (r), coefficient of determination (r^2) and standard deviation of the parameters. Kinetic parameters such as area under the curve (AUC) (equation 2), total oral clearance (equation 3) and half-lives were calculated according to Wagner[17]. Otherwise, data are presented as mean ± SE. The coefficient of correlation (r) is calculated by conventional methods. The level of significance is taken as $p < 0.05$.

$$C_p = \frac{F \cdot Dose \cdot k_a \left(e^{-k_e t} - e^{-k_a t}\right)}{V_d \left(k_a - k_e\right)} \qquad \text{(Equation 1)}$$

$$AUC = \frac{F \cdot Dose \cdot k_a}{V_d \left(k_a - k_e\right)} \left(\frac{1}{k_e} - \frac{1}{k_a}\right) \qquad \text{(Equation 2)}$$

$$\text{Total oral clearance} = \frac{F \cdot Dose}{AUC} \qquad \text{(Equation 3)}$$

RESULTS

Kinetic parameters

The plasma clonidine concentration-time data fitted well ($r^2 > 0.98$) to the biexponential function given as equation 1. The mean values of plasma clonidine concentration over the dosage interval are presented in figure 1 and the kinetic data obtained in table I. Since no kinetic evaluation of clonidine was performed after intravenous administration, the biological availability (F) is not possible to determine. The calculated volume of distribution is thus affected by F and values given in table I are in fact V_d/F. The influence of biological availability does also apply to oral clearance (equation 3). The rate constant of absorption was similar for the two dosages used with a half-life of 1.05 and 0.83 hours, respectively, yielding a maximal concentration 2-3 hours following administration. The biological half-life (t 1/2 k_e) was also about the same for the two dosages, i.e. 8.7 and 7.9 hours, respectively. The total oral clearance was 5.0 and 4.7 ml · min^{-1} · kg^{-1} and the volume of distribution (V_d/F) was 3.75 and 3.19 l · kg^{-1}. The AUC increased in relation to the dose administered.

Relationship between plasma levels of clonidine and reduction of blood pressure and pulse rate

To investigate the relation between the effect of clonidine on blood pressure and the plasma levels of the drug, the maximal percentage decrease of mean arterial blood pressure (MAP) was plotted against the logarithm of P-CLON in each patient at the two clonidine dosages. A linear relationship with a significant slope ($r = 0.91$, $p < 0.001$) was obtained in five patients (Fig. 2). Three patients showed no further decrease of MAP with an increase of plasma clonidine levels, but remained at a maximum percentage change in MAP of about 10-20%. A steady state level of 2.2-4.4 nmol/l produced a maximal change of MAP of 15-20% (15-27 mmHg). No significant relation was found between the maximal pulse rate decrease and the logarithm of P-CLON. The per cent decrease of pulse rate during clonidine was 12.6 ± 4.6% at 0.67 ± 0.08 µg/kg q.i.d. and 20.2 ± 1.7% at 1.64 ± 0.09 µg/kg q.i.d. The corresponding mean plasma clonidine concentration was 1.4 ± 0.1 nmol/l and 4.1 ± 0.6 nmol/l, respectively.

Table I. Kinetic data found after oral clonidine medication for 4-20 weeks to patients with essential hypertension. Mean ± SE

Mean dose $\mu g \cdot kg^{-1}$ BW	V_d/F $l \cdot kg^{-1}$	Absorption half life h	Biological half life h	Area under the curve $\mu g \cdot ml^{-1} \cdot min$	Total oral clearance $ml \cdot min^{-1} \cdot kg^{-1}$
0.67 ± 0.08 (n = 7)	3.75 ± 0.35	1.05 ± 0.01	8.68 ± 0.87	133.8 ± 12.6	5.0 ± 0.5
1.64 ± 0.09 (n = 8)	3.19 ± 0.33	0.83 ± 0.01	7.89 ± 0.82	350.4 ± 37.1	4.7 ± 0.5

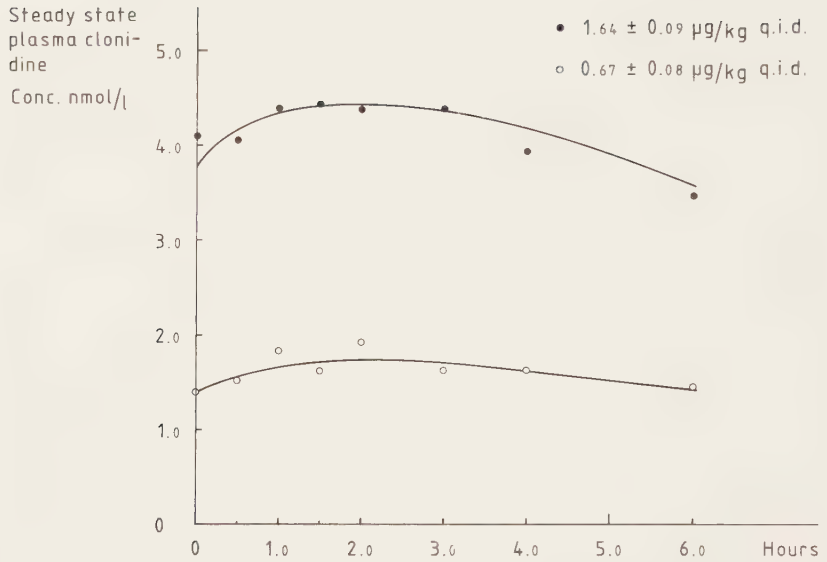

Fig. 1. Mean plasma clonidine concentration over one dosage interval of chronic clonidine treatment with two different dose levels in eight patients.

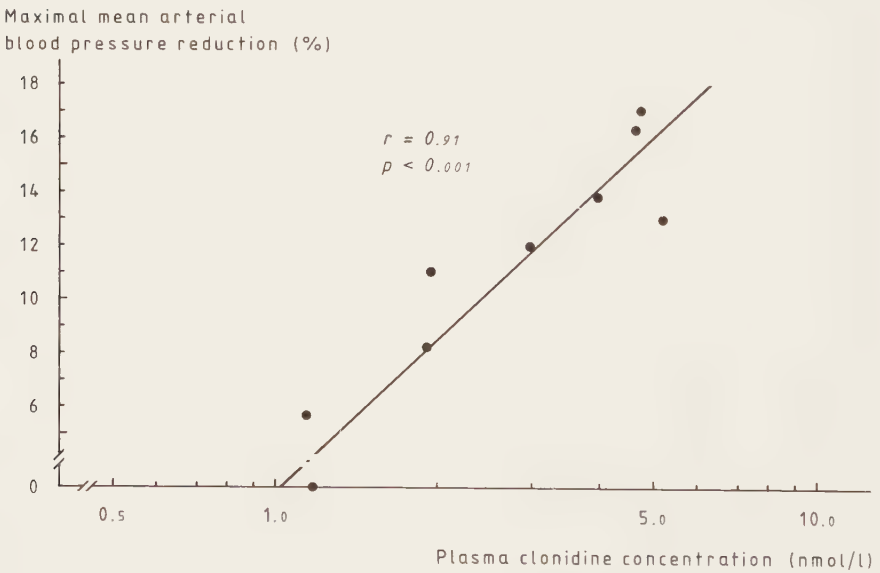

Fig. 2. Maximal reduction of mean arterial pressure (MAP) in relation to plasma clonidine after chronic treatment with two different dose levels in five patients.

Plasma levels of catecholamines and renin activity in relation to plasma levels of clonidine

A significant linear relationship was obtained between the logarithm of P-CLON and the percentage reduction of PNA ($r = 0.67$, $p < 0.01$) (Fig. 3). No relationship was found between the percentage reduction of plasma adrenaline concentration and the logarithm of P-CLON. Basal values for catecholamines are given in table II.

No significant relation was found between P-CLON and the reduction of PRA, when calculated for the whole group of patients. However, clonidine (1.64 ± 0.09 µg/kg) reduced PRA markedly in six patients (86 ± 3%) but not in two (-21 and +12%, respectively). In the former patients a significant relationship was found between the reduction (%) of PRA and the logarithm of P-CLON ($r = 0.91$, $p < 0.001$) (Fig. 4). Furthermore, in these patients a significant correlation was found between the reduction (%) of PRA and the reduction (%) of PNA ($r = 0.74$, $p < 0.01$). The relationship between the reduction (%) of PRA and the maximal percentage decrease of MAP was close to significance ($r = 0.54$, $0.1 > p > 0.05$). In the two patients demonstrating no marked decrease in PRA during clonidine treatment at the higher dose level, clonidine also caused a smaller reduction in PNA (26 and 30%, respectively) as compared to the other six patients (58 ± 6%). The two patients also showed a rather limited reduction of blood pressure (16 and 13%).

DISCUSSION

The present study demonstrates that in patients with essential hypertension given chronic clonidine treatment a significant relation exists between the fall in supine blood pressure and the plasma level of the drug. Similar relationships were previously reported in acute studies in normotensive[5] and hypertensive[8] individuals as well as after short term treatment of patients with essential hypertension[19]. The kinetic data found are also similar to the findings in acute studies, in which clonidine was given i.v.[8] and orally[5]. The obtained values of volume of distribution (V_d/F) and total oral clearance in the present investigation agreed with previous reported[5]. This indicates that the biological availability is almost complete as has been stated before[5].

Table II. Noradrenaline (PNA) and adrenaline (PA) concentrations in plasma (nmol/l) in supine position before medication in eight patients with essential hypertension

	LM	GN	BM	BG	SE	KH	RO	TT
PNA	0.4	0.6	0.7	1.5	0.8	0.7	1.2	1.1
PA	0.18	0.32	0.20	0.29	0.26	0.29	0.28	0.25

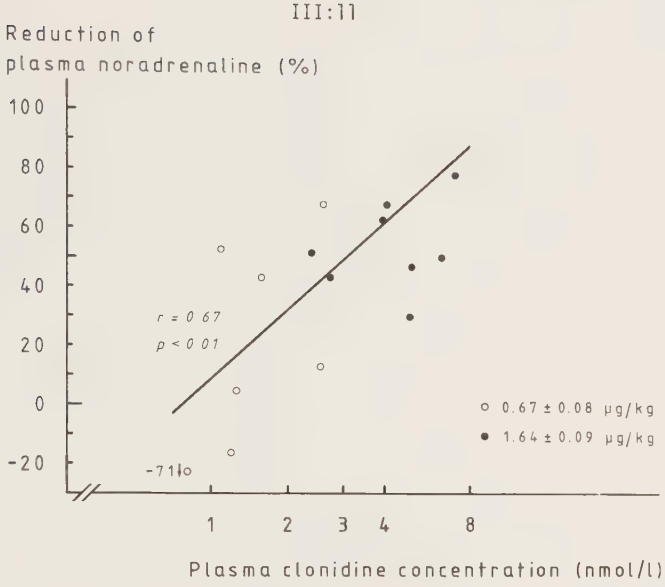

Fig. 3. Reduction of supine plasma noradrenaline in relation to plasma clonidine after chronic treatment with two different dose levels in eight patients.

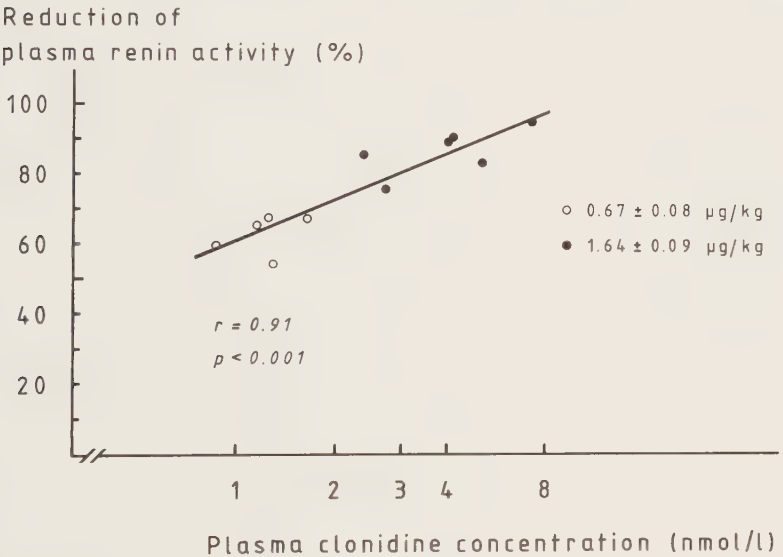

Fig. 4. Reduction of supine plasma renin activity in relation to plasma clonidine after chronic treatment with two different dose levels in six patients.

Antihypertensive drugs may have different short term as compared to long term effects. E.g. the thiazide diuretics seem to exert their initial effect on blood pressure by volume depletion[18], but by reduction of systemic vascular resistance[3] during chronic treatment. Similarly, the immediate effects of β-adrenergic blockers on systemic vascular resistance differ from those induced by chronic administration[16]. In earlier studies short term clonidine administration in man was found to reduce sympathetic nervous activity[19], and the present study demonstrates that this is also the case following long term treatment. Furthermore, as a significant correlation was found between plasma clonidine concentration and the blood pressure fall, inhibition of the sympathetic nervous activity seems to play a role for the hypotensive effect of clonidine during chronic medication. Our results are in contrast to the findings by Cohen et al.[2], who in a similar patient material found no changes in several biochemical indices of adrenergic tone after one month of clonidine treatment. These differences are not readily explained, although the final mean dosage of clonidine was higher in our study (506 µg/day) than in the study by Cohen et al.[2] (400 µg/day).

Several factors influence the release of renin (for ref. see Davis[4]), among them the renal sympathetic nerve activity and circulating catecholamines. The finding of a significant relation between P-CLON and the reduction of PRA on the one hand, and the reduction of PRA and of PNA on the other in six out of eight patients supports the view that the inhibition of the sympathetic nervous activity contributes to the reduction in PRA during clonidine medication. It seems possible that the reduction of PRA could contribute to lower blood pressure as indicated by the nearly significant relation between the decrease in PRA and the reduction in blood pressure. In the remaining two patients a higher clonidine dosage probably would have been appropriate in view of the finding that the reduction of both blood pressure and noradrenaline was smaller as compared to the other six patients. It seems possible that a higher clonidine dosage would have resulted in a further decrease in PNA and blood pressure and also a reduction of PRA.

In conclusion, the present study demonstrates that chronic clonidine treatment in patients with essential hypertension reduces supine PNA, PRA and blood pressure in relation to the plasma levels of the drug. These findings support the view that clonidine exerts its chronic antihypertensive effect at least partly by direct reduction of sympathetic nervous tone and as a consequence lowering of renin secretion.

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Long Term Treatment of Moderate Hypertension with the beta$_1$-Receptor Blocking Agent Metoprolol

II. Effect of Submaximal Work and Insulin-Induced Hypoglycaemia on Plasma Catecholamines and Renin Activity, Blood Pressure and Pulse Rate

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Summary. The effect of submaximal work and insulin-induced hypoglycaemia on plasma catecholamines and renin activity was studied in nine males with moderate hypertension before treatment, after one month on placebo and after three months of metoprolol treatment. The maintenance dose used was 50–150 mg three times daily. The placebo caused no change in blood glucose, blood pressure, pulse rate, plasma catecholamines and renin activity, neither under basal conditions nor following submaximal work or insulin-induced hypoglycaemia. Metoprolol significantly reduced blood pressure, pulse rate and plasma renin activity under basal conditions whereas plasma catecholamines were unchanged. During metoprolol treatment the increase in blood pressure and pulse rate in response to submaximal work was reduced, but the plasma noradrenaline response was enhanced and plasma adrenaline unaltered. The decrease in pulse rate after work was positively correlated with the mean plasma metoprolol concentration. The fall in blood glucose after insulin 0.1 IU/kg body weight i. v. and its return to normal was unaffected by metoprolol. Before metoprolol, hypoglycaemia was followed by a pronounced increase in plasma adrenaline, with a maximum after 45 min. During metoprolol the adrenaline increase was even more pronounced. Hypoglycaemia was also followed by a two-fold increase in plasma noradrenaline, both before and during treatment with metoprolol. Plasma renin activity during submaximal work and insulin-induced hypoglycaemia varied as much before as during treatment with metoprolol.

Key words: Beta$_1$-receptor blockade, metoprolol, hypertension, submaximal work, insulin-induced hypoglycaemia, plasma catecholamines, plasma renin.

Plasma catecholamines and renin activity normally increase in response to exercise and hypoglycaemia [1–5]. The renin response seems to be related to enhanced sympathetic activity [2]. In a previous study the non-selective beta-receptor blocking agent penbutolol was found to increase the catecholamine response to exercise, but markedly to reduce the adrenaline response following hypoglycaemia [6, 7]. Penbutolol also suppressed plasma renin activity under basal conditions, during exercise and after hypoglycaemia [6, 7]. In this paper the effect of the cardioselective beta-receptor blocking agent metoprolol on these mechanisms is presented

Material and Methods

Studies were performed in nine males with moderate hypertension. Two patients had quite low plasma renin activity, four had intermediate levels and three had comparatively high values in the supine position. Further clinical data have been published previously [8].

During the studies the patients were hospitalized in a metabolic ward for 8–10 days where they received a diet containing sodium 120 mEq and potassium 95 mEq daily. All studies were performed in an identical manner before treatment, after one month on placebo and after about three months on metoprolol. Treatment with metoprolol was started with 25 mg three times daily and the dose was gradually increased to 50–150 mg given at 7 a. m., 2 p. m. and 10 p. m. and maintained for 4–17 weeks.

All patients were subjected to a standardized submaximal work test consisting of exercise with the patient seated on a bicycle ergometer. The load used was determined in advance for each patient without any kind of medication; it was defined as the work load causing a steady state pulse rate of approxi-

Table 1. Plasma noradrenaline (ng/100 ml) during submaximal work before and during placebo and metoprolol treatment

Patient	No treatment			Placebo			Metoprolol		
	Before work	After work	Absolute change	Before work	After work	Absolute change	Before work	After work	Absolute change
I	99	–	–	77	212	155	128	387	259
II	–	84	–	52	83	32	60	142	83
III	65	147	82	53	108	54	82	118	37
IV	111	145	34	94	145	51	67	166	98
V	72	237	165	74	236	162	139	376	237
VI	124	228	104	92	140	48	107	194	87
VII	117	140	23	99	142	43	61	255	193
VIII	252	329	77	132	214	82	128	558	429
IX	121	197	76	80	221	141	176	418	242
Mean ± SD	120.0 ±57.6	188.3 ±76.2	80.2 ±46.8	83.5 ±24.6	166.7 ±55.2	83.2 ±49.5	105.4 ±40.6	290.4 ±150.9	185.1 ±122.9

Table 2. Plasma adrenaline (ng/100 ml) during submaximal work before and during placebo and metoprolol treatment

Patient	No treatment			Placebo			Metoprolol		
	Before work	After work	Absolute change	Before work	After work	Absolute change	Before work	After work	Absolute change
I	9.2	–	–	6.3	4.8	−1.5	11.7	12.6	0.9
II	–	7.7	–	6.7	9.6	2.9	9.3	9.7	0.2
III	8.9	18.4	9.5	11.4	9.5	−1.9	10.6	19.5	8.9
IV	9.2	12.3	3.1	8.6	12.3	3.7	9.4	13.1	3.7
V	9.1	13.5	4.4	10.4	12.2	1.8	15.9	16.4	0.5
VI	13.9	30.5	16.6	15.9	16.8	0.9	20.3	29.9	9.6
VII	15.2	12.0	−3.2	21.1	27.4	6.3	13.9	31.9	18.0
VIII	9.4	11.2	1.8	5.5	8.0	2.5	8.0	13.1	5.1
IX	19.8	13.3	−6.5	8.6	14.2	5.6	13.7	14.7	1.0
Mean±SD	11.8±4.1	14.9±7.0	3.7±7.7	10.5±5.1	12.8±6.5	2.3±2.8	12.5±3.9	17.9±7.9	5.3±5.9

mately 130/min. This predetermined work load was employed for six minutes under all three conditions studied. It was always performed at noon after 8–10 days of hospitalization, except for Patient I who was tested after 5 days of placebo.

Pulse rate and blood pressure (mercury manometer) were recorded immediately before bicycling and once every minute during the work test. The figures for pulse rate and blood pressure "after work" refer to measurements immediately after completion of the work test.

Blood samples for determination of pre-exercise control values of plasma catecholamines and renin activity were collected at noon on two consecutive days following 30 min of walking around the ward prior to the work test; the pre-exercise values given in Tables 1–3 represent the means of two determinations. Further blood samples for estimation of plasma catecholamines and renin activity were collected immediately after completion of the work test. In Patient I and II, three of the blood samples taken before treatment for analysis of catecholamines were lost due to a technical error.

The patients were fasted and confined to bed from 10 p. m. on the day prior to the insulin test. Crystalline insulin (Vitrum) was injected intravenously at 8 a. m., at the dose of 0.1 IU/kg body weight.

Blood samples for determination of blood glucose, plasma catecholamines and renin activity were taken from an antecubital vein 10 min and immediately before the insulin injection, and then after 30, 45, 60 and 120 min. Pulse rate and blood pressure (mercury manometer) were recorded at the same times.

Blood glucose was determined by a glucose oxidation method [9, 10]. A double isotope derivative technique was employed for assay of catecholamines [11]. Plasma renin activity was determined by the method of Haber et al. [12], using a radioim-

Table 3. Plasma renin activity (ng/100 ml) during submaximal work before and during placebo and metoprolol treatment

Patient	No treatment			Placebo			Metoprolol		
	Before work	After work	Absolute change	Before work	After work	Absolute change	Before work	After work	Absolute change
I	29	106	77	106	151	45	13	12	−1
II	670	1441	771	667	1350	683	937	2613	1676
III	181	320	139	155	308	153	8	17	9
IV	262	347	85	277	465	188	212	437	225
V	399	1148	749	477	789	312	332	453	121
VI	874	1419	545	815	1179	364	322	782	460
VII	1229	1328	99	823	1304	481	198	785	587
VIII	357	1349	992	522	1276	754	216	1072	856
IX	171	324	153	102	235	133	19	60	41
Mean±SD	464±388	865±570	401±363	438±292	784±502	346±249	251±287	692±813	442±548

munoassay for the estimation of angiotensin I produced after incubation for three hours. The entire series of blood samples collected in each test was analyzed in a single assay run for catecholamines and renin activity, respectively. In Patient I technical error led to loss of the six blood samples collected for determination of catecholamines during insulin-induced hypoglycaemia.

Control studies, in which physiological saline 0.3 ml was injected instead of insulin, were performed in all patients both before treatment, during placebo and during metoprolol (except Patient I during placebo).

Data presented in the text are given as means ± SD; arithmetic means standard deviations and correlation coefficients were calculated by conventional formulae. Statistical significance of differences was assessed by Student's t-test for paired data. A difference was considered significant when $p < 0.05$.

Results

Placebo had no effect on any of the parameters studied, neither under basal conditions nor following submaximal work or insulin-induced hypoglycaemia.

Before metoprolol submaximal work caused an increase in systolic blood pressure from 139 ± 9 to 186 ± 18 mm Hg and in pulse rate from 89 ± 13 to 132 ± 9/min, whereas diastolic pressure remained unchanged (97 and 99 respectively). Metoprolol reduced pre-exercise blood pressure (115/81 ± 13/11; $p < 0.005$), pulse rate (63 ± 10; $p < 0.005$), blood pressure (147/83 ± 18/11; $p < 0.01$) and pulse rate (98 ± 13; $p < 0.001$) following submaximal work. During metoprolol the percentage decrease in maximal pulse rate was correlated with the mean plasma metoprolol concentration ($r = 0.81$, $p < 0.01$).

The pre-exercise levels of noradrenaline and adrenaline under control conditions and following metoprolol ($p > 0.05$) (Tables 1 and 2) were similar.

During metoprolol the noradrenaline response to exercise was significantly higher than before treatment ($p < 0.02$; Table 1), whereas the adrenaline response was variable ($p > 0.05$; Table 2).

The pre-exercise values for plasma renin activity were significantly reduced following treatment with metoprolol ($p < 0.025$). Before metoprolol, plasma renin activity increased in all patients in response to work (Table 3), but during metoprolol the plasma renin response to work was clearly reduced in four patients (I, III, V and IX), whereas other patients showed similar responses before and during metoprolol. The ratio between the exercise-induced increase in plasma renin activity during metoprolol and placebo was inversely correlated with the mean plasma metoprolol concentrations ($r = -0.73$, $p < 0.025$).

During metoprolol no consistent changes were observed in fasting blood glucose ($p > 0.05$; Table 4), or in basal plasma catecholamine levels ($p > 0.05$; Table 5, Fig. 1) but there was a significant decrease in basal plasma renin activity ($p < 0.005$; Table 6), blood pressure ($p < 0.05$; Fig. 1) and pulse rate ($p < 0.005$; Table 4).

After insulin, blood glucose fell more than 50 per cent after 30 min and returned to normal within a further 90 min under all three conditions studied (Table 4).

Before metoprolol hypoglycaemia was followed by a pronounced increase in plasma adrenaline in all patients, with a maximum at 45 min after the injec-

Table 4. Blood glucose (mg/100 ml) and pulse rate in Patients I–IX during insulin-induced hypoglycaemia before treatment (BT) and during placebo (Pl) and metoprolol (M) treatment

Time in min		−10	0	30	45	60	120
Glucose Mean ± SD	BT	71±5	73±6	27±9	42+7	51±8	72±6
	Pl	65±9	68±11	29±11	36+8	49±10	68±6
	M	70±6	73±6	27±6	38+6	51±7	69±7
Pulse rate Mean ± SD	BT	63±7	62±7	70±9	69±7	67±8	62±6
	Pl	63±12	62±8	67±11	67±8	63±7	60±8
	M	53±8	51±8	53±10	54±9	51±6	50±7

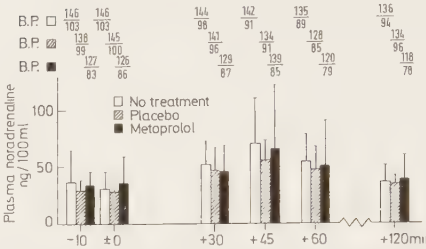

Fig. 1. Blood pressure (means) and plasma noradrenaline (means ± SD) during insulin-induced hypoglycaemia before and during metoprolol

Table 5. Plasma adrenaline (ng/100 ml) during insulin-induced hypoglycaemia before treatment (BT) and during placebo (Pl) and metoprolol (M) treatment

Time in min		−10	0	30	45	60	120
Pat I	BT	–	–	–	–	–	–
	Pl	3.0	2.7	36.6	29.4	10.5	5.4
	M	3.4	3.4	30.5	132.7	44.0	11.3
Pat II	BT	3.1	3.0	15.7	61.9	26.6	8.6
	Pl	2.1	3.0	4.7	60.9	13.7	5.5
	M	2.7	3.1	147.4	179.1	54.9	18.3
Pat III	BT	7.6	5.8	12.8	39.5	22.4	9.0
	Pl	6.3	4.8	8.7	39.7	18.8	11.7
	M	5.5	4.0	10.1	65.0	37.5	14.9
Pat IV	BT	3.7	4.7	21.3	67.8	29.2	11.4
	Pl	4.4	3.1	30.9	70.9	37.5	13.9
	M	4.6	4.5	14.4	102.4	53.9	17.6
Pat V	BT	3.1	2.7	3.4	7.3	15.8	4.6
	Pl	3.2	2.9	4.0	43.8	17.9	5.3
	M	4.0	4.9	3.9	51.7	53.9	8.1
Pat VI	BT	8.6	9.9	106.8	146.2	23.8	16.7
	Pl	4.3	7.6	61.4	73.9	34.1	11.1
	M	6.5	4.7	41.0	208.6	37.6	13.0
Pat VII	BT	4.2	5.1	21.6	32.5	29.9	21.9
	Pl	6.2	4.5	10.4	41.7	23.9	14.5
	M	5.6	5.5	7.8	90.7	31.9	11.6
Pat VIII	BT	4.7	5.3	7.0	25.9	28.2	10.1
	Pl	3.6	3.9	3.9	77.9	28.6	6.5
	M	4.8	5.7	8.7	214.6	57.5	15.6
Pat IX	BT	2.9	3.4	21.1	65.0	34.3	9.4
	Pl	2.6	3.2	26.1	48.4	35.6	6.1
	M	3.7	2.7	3.7	6.1	8.0	3.4
Mean ± SD	BT	4.7±2.2	5.0±2.3	26.2±33.3	55.8±42.2	26.3±5.6	11.5±5.4
	Pl	4.0±1.5	4.0±1.5	20.7±19.7	54.1±17.3	24.5±9.9	8.9±3.9
	M	4.5±1.2	4.3±1.1	29.7±45.9	116.8±72.6	42.1±15.8	12.6±4.7

tion of insulin (Table 5). The adrenaline response varied considerably from one patient to another. Treatment with metoprolol was followed by a more pronounced increase in the adrenaline response to hypoglycaemia measured after 45 ($p < 0.02$) and 60 min ($p < 0.05$). Of the nine patients studied, one (IX) was unique in demonstrating a decreased adrenaline response to hypoglycaemia during metoprolol.

Hypoglycemia was also accompanied by a two-fold rise in plasma noradrenaline, which also reached its maximum 45 min after the injection of insulin (Fig. 1). The noradrenaline response was similar before and during treatment with metoprolol ($p > 0.05$).

The effect of hypoglycaemia on plasma renin activity varied (Table 6). Before metoprolol, hypoglycaemia caused a definite increase in plasma renin activity in four of the patients (III, IV, VI, VII). In three patients (I, II, IX) plasma renin activity in-

Table 6. Plasma renin activity (ng/100 ml) during insulin-induced hypoglycaemia before treatment (BT) and during placebo (Pl) and metoprolol (M) treatment

Time in min		−10	0	30	45	60	120
Pat I	BT	8	45	15	15	30	15
	Pl	35	38	61	59	42	57
	M	6	5	8	6	6	3
Pat II	BT	344	572	450	510	1198	431
	Pl	344	317	338	400	401	411
	M	214	176	245	428	823	505
Pat III	BT	174	151	214	219	245	307
	Pl	152	117	163	170	207	193
	M	4	20	2	39	9	12
Pat IV	BT	112	57	84	196	184	97
	Pl	121	99	157	241	169	157
	M	39	30	46	116	86	43
Pat V	BT	380	314	380	357	392	330
	Pl	276	273	241	270	321	289
	M	274	185	231	208	357	314
Pat VI	BT	122	101	184	314	318	329
	Pl	84	74	135	214	318	350
	M	58	63	72	221	202	101
Pat VII	BT	208	193	262	305	305	348
	Pl	135	163	208	268	336	314
	M	70	81	80	66	86	84
Pat VIII	BT	257	–	283	235	183	243
	Pl	187	151	157	161	196	184
	M	99	75	69	64	66	45
Pat IX	BT	36	31	64	72	84	77
	Pl	34	39	40	43	40	40
	M	18	18	22	13	10	15
Mean±SD	BT	182±128	183±183	215±146	247±149	327±346	242±144
	Pl	152±104	141±98	167±90	203±111	226±129	222±128
	M	87±95	73±67	86±91	129±137	183±266	125±171

creased in only one of the two control studies before metoprolol. The remaining two patients (V, VIII) showed no response in any of the control studies. During treatment with metoprolol, plasma renin activity did not change in response to hypoglycemia in four patients (I, VII, VIII, IX), but an increase was seen in five (II, III, IV, V, VI).

Prior to metoprolol treatment hypoglycaemia resulted in a fall both in systolic and diastolic blood pressure at 45, 60 and 120 min after the injection of insulin ($p < 0.05$; Fig. 1). During metoprolol systolic blood pressure increased ($p < 0.005$), whereas diastolic pressure was unchanged at 45 min ($p > 0.05$) after insulin, and then there was a fall in both systolic and diastolic blood pressures.

Hypoglycaemia before treatment was accompanied by a significant increase in pulse rate ($p < 0.01$), but this did not occur in the placebo or metoprolol periods ($p > 0.05$; Table 4).

In the control studies blood glucose was 71 ± 6 mg/100 ml before the injection of physiological saline and it increased gradually to 79 ± 5 at 120 min after the injection ($p < 0.005$), both before medication and during metoprolol. Plasma catecholamines, plasma renin activity, blood pressure and pulse rate were not affected by the saline injection.

Discussion

Non-selective beta-receptor blocking drugs effectively lower both the blood pressure response and the increase in pulse rate normally seen following exercise [13–15]. The results of the present investigations indicate that the cardioselective beta-receptor blocking drug metoprolol has similar properties.

Under certain conditions physical exercise leads to an increase in plasma noradrenaline. The degree of the noradrenaline response to work is, however, related to the intensity of the exercise performed [2, 4]. A work load sufficiently heavy to provoke an increase in pulse rate of at least 30 beats/min appears to be the minimum required to elicit a measurable increase in plasma noradrenaline [4]. Very heavy exercise leads also to an increase in plasma adrenaline [2–4]. In the present studies submaximal work induced a moderate increase in plasma noradrenaline concentrations before treatment and during placebo. In an earlier study submaximal work caused no change in plasma noradrenaline in untreated hypertensive patients [6]. This discrepancy can probably be explained by the fact that the work load applied was heavier in the present studies, in which the patients showed a lower pre-exercise

pulse rate without medication and accordingly had a more pronounced increase in pulse rate when subjected to submaximal work as defined above (130 beats/min).

In earlier studies the blood levels both of noradrenaline and adrenaline were found to increase in response to exercise during treatment with non-selective beta-receptor blocking drugs [6, 16]. In the present studies with the cardioselective beta-receptor blocking agent metoprolol, the noradrenaline response to work increased similarly, whereas adrenaline showed no consistent change. It is generally agreed that work requires an increase in sympathetic activity and it seems probable that the excessive increase in noradrenaline response under the present conditions was necessary to compensate for the receptor-blocking effects of metoprolol. The finding that during treatment with metoprolol there was no increase in adrenaline following work, whereas there was a clear increase during medication with the non-selective beta-receptor blocking agent penbutolol, might be related to the fact that stimulation of beta$_1$-receptors is mediated mainly via noradrenaline, whereas stimulation of beta$_2$-receptors is mediated by adrenaline [17].

The possibility that the increased blood levels of noradrenaline following work in the metoprolol-treated patients depend on a reduction in catecholamine extraction by the liver, cannot be excluded [18]. Another possibility is related to the recent finding that sympathetic nerve endings contain beta-receptors which can influence noradrenaline release by a feed-back mechanism [19–22], and that propranolol in high concentrations increases the output of noradrenaline that normally follows nerve stimulation [19]. This property of high concentrations of propranolol has been ascribed to inhibition of the re-uptake of noradrenaline in the nerve endings [19]. Metoprolol might act in a similar manner.

The markedly increased noradrenaline response to exercise during treatment with beta-receptor blocking drugs might have functional consequences, because it could lead to overstimulation of the alpha-receptors engaged in regulation of circulatory and metabolic processes.

It is well established that hypoglycaemia normally leads to increased secretion of adrenaline from the adrenal medulla, and that this is mediated via a central nervous mechanism [5, 7, 23–28]. We have previously reported that treatment with the non-selective betareceptor blocking agent penbutolol is followed by a diminshed adrenaline response to hypoglycaemia, a finding which could be interpreted as circumstantial evidence for the presence of a beta-receptor-mechanism or mechanisms in the brain [7]. It is of interest that various non-selective beta-receptor blocking agents, and the cardioselective beta-receptor blocking agent metoprolol, have been shown to penetrate into the brain [29–31]. The finding that metoprolol, unlike penbutolol, is accompanied by an increased adrenaline response to hypoglycaemia would then indicate that a beta$_2$-receptor meachnism located in the central nervous system was involved in the hypoglycaemia-induced adrenaline response. Further support for the engagement of adrenergic mechanisms in the adrenaline response to hypoglycaemia was reported previously by Hedeland et al. [27], who found an inhibitory effect on it of clonidine.

During hypoglycaemia increased amounts of glucose are produced partly via glycogenolysis in the liver and partly by utilization in the liver of glycerol produced by peripheral lipolysis. It is well documented that beta$_1$-receptors are engaged in the liberation of free fatty acids [17, 32] and that beta-receptors (of unclassified type) are also involved in glycogenolysis [33]. Our finding that the markedly increased adrenaline response to hypoglycaemia during metoprolol did not result in a faster return of blood glucose to normal levels might indicate that beta$_1$-receptors were of importance for glucose production during hypoglycaemia. Earlier reports that the non-selective beta-receptor blocking agent propranolol caused a delay in the return of blood glucose following insulin-induced hypoglycaemia gives additional support to this concept [34].

Renin release is partly under sympathetic control [35–41]. Exercise normally leads to an increase in renin activity [1–3], which is related to enhanced sympathetic activity [2]. Insulin-induced hypoglycaemia can lead to an increase in plasma renin activity, especially in patients with normal or high basal plasma renin [5, 7, 26, 27, 42–43]. The hypoglycaemia-induced renin response has been attributed to high levels of circulating catecholamines [5, 7] and/or to sympathetic reflex stimulation at the kidney level [42]. It can be completely abolished by treatment with non-selective beta-receptor blocking drugs [7, 26, 42], and markedly reduced by clonidine [27]. Similar studies in man however have shown that insulin-induced hypoglycaemia in many instances is not followed by an increase in plasma renin activity [7, 27–28, 43]. This has been noted particularly in hypertensive patients with a low level of plasma renin activity [7, 27, 43].

The finging that metoprolol, like non-selective beta-receptor blocking drugs, reduces plasma renin activity in the supine and upright positions supports earlier reports with other beta$_1$-receptor blocking agents [44–45]. In our studies with metoprolol the renin response to work was almost completely abolished in four of the patients, no clear difference was observed in four others and there was a marked increase in one. These divergent results cannot be

explained by differences in the catecholamine response to work, because some patients with a marked increase in plasma catecholamines showed a clear reduction in the renin response, whereas the opposite combination was seen in others (Tables 1, 2).

The studies of the response of renin activity to hypoglycaemia have provided a further illustration of the variable sensitivity of the renin mechanism to sympathetic stimulation. The patients with the lowest basal renin activity showed no or only a minimal increase in renin activity after insulin, but three patients with comparatively high basal renin activity also demonstrated a similar pattern. The reason for this lack of response is unclear, but the studies seem to demonstrate that it was not due to absence of the catecholamine response to insulin-induced hypoglycaemia. Non-selective beta-receptor blocking compounds very effectively inhibit renin release in response to work and hypoglycaemia, respectively. Some authors maintain that renin release is under the control of $beta_1$-receptors [46], but others believe that the sympathetic influence on renin release is mediated via $beta_2$-receptors [47, 48]. The renin response to isoproterenol is unaffected by $beta_1$-receptor blockade with practolol [47]. The finding that basal renin activity was suppressed during metoprolol indicates that renin release is partly mediated by $beta_1$-receptor mechanisms. As hypoglycaemia leads to a pronounced increase in plasma adrenaline, which has much higher affinity for $beta_2$- than $beta_1$-receptors [17], these results support the idea that renin release may also be mediated by $beta_2$-receptor stimulation.

At the same time as the high circulating levels of adrenaline in hypoglycaemia, a clear fall in blood pressure occurred when the patients were without medication or taking placebo. This decrease in blood pressure was probably due to stimulation of $beta_2$-receptors in vascular smooth muscle. During metoprolol, on the other hand, high circulating adrenaline was associated with a temporary increase in systolic blood pressure, probably due to overstimulation of alpha-receptors. To judge from the subsequent fall in blood pressure, however, this mechanism was probably overcome, possibly as a result of stimulation of the unblocked $beta_2$-receptors. Thus, metoprolol seems to have effects both similar to and different from those of propranolol, which has been found to induce a persistent increase in blood pressure during infusion of adrenaline [49].

There were only minimal changes in heart rate after hypoglycaemia both before treatment and during placebo, despite the very high adrenaline levels. This is in agreement with previous reports [7, 50] and again illustrates the low affinity of adrenaline for $beta_1$-receptors [17]. The small increase in heart rate observed in some patients might be explained by a reflexogenic increase in cardiac sympathetic nervous activity due to peripheral vasodilatation.

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The effect of captopril on catecholamines, renin activity, angiotensin II and aldosterone in plasma during physical exercise in hypertensive patients

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Abstract. The studies were designed to explore the effect of the converting enzyme inhibitor captopril on the activity of the sympathetic nervous system during basal conditions and following graded physical exercise in patients with essential hypertension. Seven males and two females, aged 36–59 years, were hospitalized under metabolic ward conditions and treated for 7 days with captopril given orally in increasing dosages, the final dose being 600 mg daily. The patients were subjected to an individual, graded submaximal work test (bicycling) for 20 min before medication and then again in an identical manner during medication with 600 mg captopril. Blood samples were drawn before exercise and then after 10 and 20 min of work for the determination of plasma angiotensin II (PA II), plasma aldosterone (PAC), plasma renin activity (PRA), plasma noradrenaline (PNA) and plasma adrenaline (PA). Before medication blood pressure (mmHg) was 195/133 immediately before exercise, 230/129 after 10 min of moderate exercise and 263/105 following 20 min of nearly maximal work. During treatment with captopril the respective blood pressure values were 154/110, 200/100 and 245/98. Captopril had no significant effect on the changes in heart rate following physical exercise. PA II and PAC were substantially reduced and PRA considerably increased by captopril. PA II, PAC and PRA increased in response to exercise both before and following captopril. The exercise stimulated increase in PNA and PA was almost identical before and during captopril. Thus, captopril had no major effect on the activity of the sympathetic nervous system in patients with essential hypertension, neither during basic conditions nor during heavy physical exercise in spite of a profound decrease in PA II.

Key words. Aldosterone, angiotensin II, captopril, catecholamines, exercise, hypertension.

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Introduction

Experimental studies in various species, including man, have demonstrated that angiotensin II can stimulate the sympathetic nervous system at the central [1–3] as well as the peripheral level [4–6]. Another approach for investigating the functional interrelationship between the renin–angiotensin system and the sympathetic nervous system is to reduce angiotensin II formation by inhibition of converting enzyme. Some such studies have been reported in hypertensive individuals [7–9], but mainly under basal conditions. In the present study the effect of angiotensin converting enzyme inhibitor captopril on angiotensin II, renin activity and aldosterone in plasma on the one hand, and plasma catecholamines on the other, was investigated both under basic conditions and during exercise in patients with essential hypertension.

Material and Methods

Patients

The patient material consisted of seven men and two women, aged 35–58 years, with essential hypertension (Table 1). Eight were classified as WHO stage I–II and one as stage III. No antihypertensive treatment was given for at least 3 weeks prior to the study.

Design of the study

The patients were admitted to a metabolic ward for 11 days. They were maintained on a diet containing 120 mmol of sodium and 95 mmol of potassium daily. Captopril was administered from day 5 in divided doses at 07.00, 15.00 and 23.00 hours, the total dose being 75 mg on day 5, 150 mg on day 6, 300 mg on day 7–8 and 600 mg on day 9–11.

On day 1 the maximal working capacity (MWC) was measured in each individual patient. The test was performed in the sitting position on an electrically braked bicycle ergometer (Elema), the work load being continuously increased with 10 W/min as described by

Table 1. Clinical and laboratory findings in nine patients studied

No.	Age	Sex	Body weight and height (kg/cm)	Supine ambulatory blood pressure (mmHg)	Eye ground KW	Cardiac size (ml/m^2)	ECG	Serum creatinine (μmol/l)	I.v. pyelography	Renal angiography
I	45	F	78/161	210/110	I	400	Normal	87	Normal	—
II	49	F	69/157	170/120	II	490	Normal	81	Normal	Normal
III	36	M	79/173	185/115	I	370	LVH	100	—	Normal
IV	45	M	92/176	190/120	II	515	LVH	99	—	Normal
V	59	M	76/176	200/130	II	440	Normal	94	—	Normal
VI	37	M	73/180	210/135	I	440	LVH	100	Normal	Normal
VII	36	M	74/186	170/115	I	340	LVH	109	Normal	Normal
VIII	46	M	82/172	200/115	II	410	Normal	76	Normal	Normal
IX	55	M	79/172	230/135	II	420	LVH	133	Normal	Nephrosclerosis
Normal range								60–115		

Åström & Jonsson [10]. The initial load applied was 50 W in the female patients and 70 W in the males. This load was maintained for 4 min before starting the load increase. The patients then continued working until exhaustion. The electrocardiogram was recorded continuously and heart rate counted from the electrocardiogram at the end of each period.

On the basis of the results obtained from MWC, the patients were subjected to an individual graded work test at 10.00 hours on day 3 (before captopril) and then repeated in an identical manner on day 11 (during captopril). During this test the patients bicycled for 10 min at a work load of 40% of their respective MWC (moderate exercise), immediately followed by an increase in the work load to 70% of MWC (submaximal exercise). This work load was applied for 10 min in six patients, whereas three patients were unable to continue beyond 6 min (patients I, II and VII).

Blood pressure was recorded immediately before the work test when the patients had been seated quietly on the bicycle for 10 min and then every second minute during exercise. The blood pressure readings were performed by one of the investigators using a sphygmomanometer and diastolic values were taken at Korotkoff phase IV. Heart rate was registered continuously by electrocardiography. The level of exhaustion at the end of the work test was subjectively rated by the patients using a 10-graded scale [11].

Blood samples for the determination of catecholamines, angiotensin II, aldosterone and renin activity in plasma were drawn through an indwelling catheter (Venflon, Viggo, Helsingborg, Sweden) kept patent by a slow saline infusion. Samples were taken immediately before exercise, after 10 min on 40% of MWC, and after 10 (6) min on 70% of MWC. The samples were immediately chilled on ice, plasma separated and stored at −20°C until assayed. All samples from the same individual were determined in one and the same assay run. The three blood samples taken after 6 min on 70% of MWC are presented together with the six samples taken after 10 min of 70% of MWC.

Analytical methods

Plasma noradrenaline and adrenaline were determined using a single isotope radioenzymatic technique [12]. The intra-assay coefficient of variation (ICV) for noradrenaline was 11% when measured at a range of 0·7–8·5 nmol/l and for adrenaline 15% (range 0·1–4·8 nmol/l). Plasma renin activity was determined as the amount of angiotensin I generated during 3 h incubation at pH 6·0 [13, 14], ICV = 7·7% (range 1·2–12·4 μg/l/3 h). Plasma angiotensin II was extracted on Dowex AG 50 W-2 resin, and determined by radioimmunoassay [15], ICV = 8% (range 3–60 pg/ml). Plasma aldosterone was determined by radioimmunoassay [16], ICV = 5% (range 1·1–21·2 nmol/l).

Statistical evaluation

Statistical analysis was performed by non-parametric methods. Data presented are given as median values and ranges. The statistical significance of differences was assessed by the Wilcoxon's two-sample-test for paired observations and Spearman's rank correlation was used to calculate the correlation coefficient (R). The level of significance was taken as $P < 0{\cdot}05$

The investigations were approved by the Ethical Committee of the Medical Faculty, University of Lund. Informed consent was obtained from all patients.

Results

Captopril significantly lowered systolic and diastolic blood pressure before exercise and during exercise at a work load of 40% of MWC (Table 2 and Fig. 1). At 70% of MWC systolic blood pressure was significantly reduced only after 4 min, and diastolic blood pressure after 2, 4 and 6 min of exercise (Fig. 1).

When the patients stopped working they rated the level of exhaustion as 8 (6–9) before captopril and 7·6 (6–9) during captopril ($P > 0{\cdot}05$).

Table 2. Blood pressure (BP) (mmHg) and heart rate (beats/min) in connection with bicycle ergometer exercise for 20 min before medication and following captopril for 7 days. The work load was 40% of maximal working capacity (MWC) for the first 10 min and 70% of MWC for the last 10 min. Median values and ranges are given.

	0 min	2 min	4 min	6 min	8 min	10 min	2 min	4 min	6 min	8 min	10 min
Before captopril											
Systolic BP	195 (175–230)	224 (180–245)	230 (190–245)	225 (215–250)	225 (210–260)	230 (200–270)	241 (210–255)	245 (210–270)	253 (220–270)	259 (230–265)	263 (245–265)
Diastolic BP	133 (115–135)	125 (105–145)	125 (110–145)	127 (120–140)	124 (110–135)	129 (115–140)	123 (95–140)	122 (100–140)	122 (120–135)	110 (100–135)	105 (95–135)
Heart rate	95 (57–112)	120 (88–130)	124 (88–130)	124 (90–132)	124 (88–134)	129 (84–144)	140 (102–156)	152 (120–168)	160 (122–174)	158 (126–184)	159 (128–168)
During captopril											
Systolic BP	154 (125–180)	183 (175–250)	205 (180–260)	202 (190–260)	208 (180–255)	200 (180–250)	225 (200–265)	236 (210–280)	250 (235–280)	245 (235–260)	245 (230–260)
Diastolic BP	110 (90–125)	110 (90–125)	104 (90–120)	100 (95–135)	97 (95–135)	100 (90–115)	110 (85–120)	106 (95–130)	100 (95–115)	105 (75–115)	98 (70–115)
Heart rate	94 (55–127)	118 (82–150)	124 (84–150)	126 (83–160)	126 (87–162)	136 (82–168)	150 (108–200)	158 (118–196)	162 (123–182)	161 (128–180)	160 (130–176)
No.	9	9	9	9	9	9	9	9	9	6	6
Exercise	← 40% of MWC →						← 70% of MWC →				

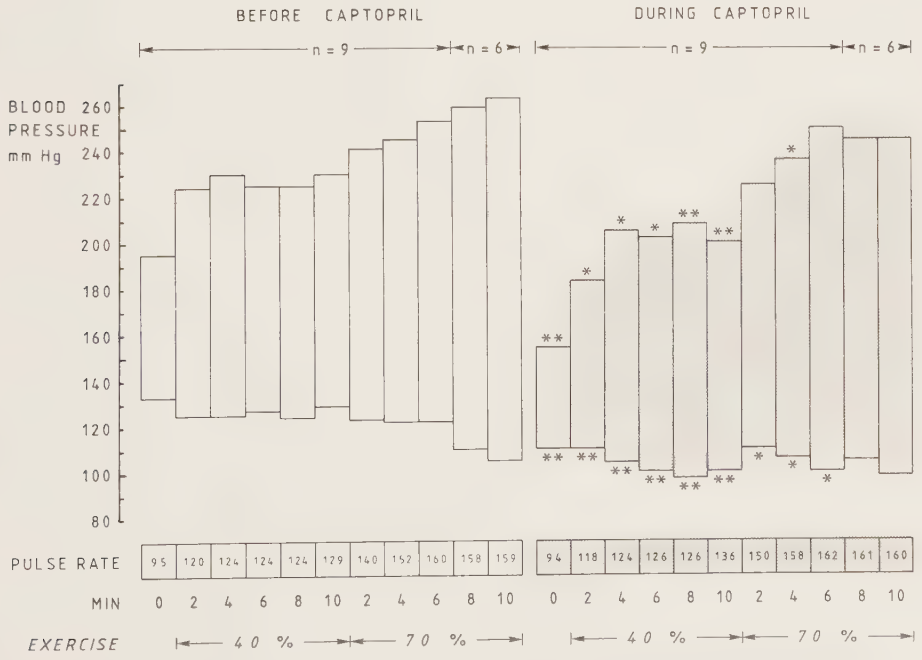

Figure 1. Blood pressure and heart rate at bicycle ergometer exercise for 20 min before medication and following 7 days of captopril treatment. The work load was for the first 10 min 40% of maximal work capacity (MWC) and for the last 10 min 70% of MWC. * Denotes $P < 0.05$, ** denotes $P < 0.01$.

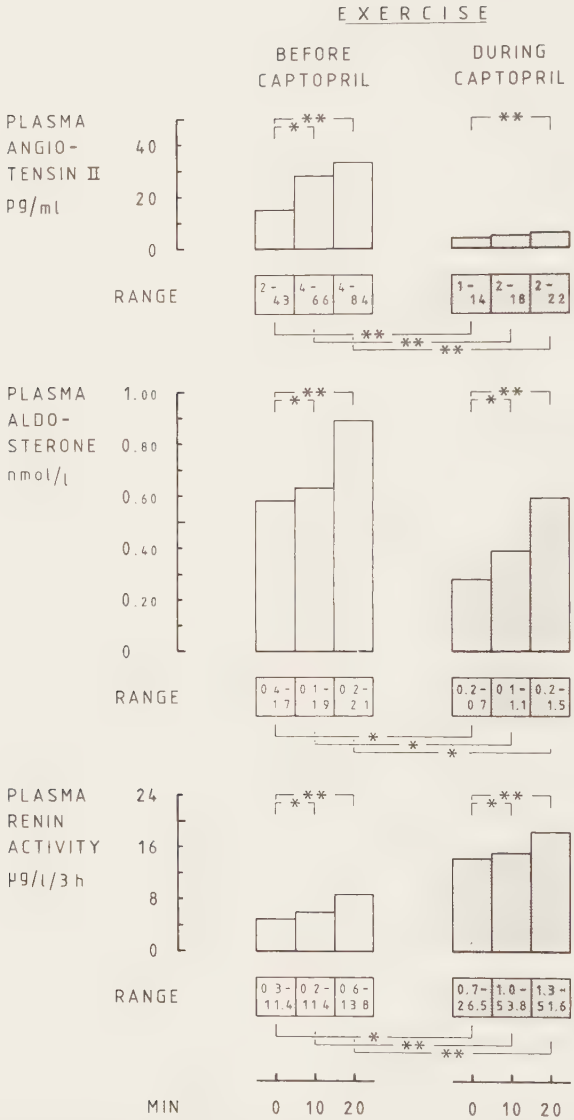

Figure 2. The response of plasma angiotensin II, plasma aldosterone and plasma renin activity to graded bicycle ergometer exercise before medication and following 7 days of captopril treatment. * Denotes $P < 0{\cdot}05$, ** denotes $P < 0{\cdot}01$.

Plasma angiotensin II concentration (PA II) was markedly reduced by captopril before exercise (pre-exercise) (Fig. 2). In response to exercise PA II increased both before and during medicaton, but the increase was less pronounced during captopril, although still significant.

Pre-exercise plasma aldosterone concentration (PAC) was reduced during captopril. During exercise PAC increased both before and during medication (Fig. 2), but PAC was lower during captopril than before medication.

Pre-exercise plasma renin activity (PRA) increased substantially following captopril (Fig. 2). Physical exercise resulted in an increase in PRA, which was more pronounced during captopril than before medication.

The pre-exercise concentrations of noradrenaline (PNA) and adrenaline (PA) in plasma were the same before and during captopril (Fig. 3). Similarly, the exercise induced changes in the plasma catecholamines were almost identical before and during medication.

Figure 3. The response of plasma noradrenaline and plasma adrenaline to graded bicycle ergometer exercise before medication and following 7 days of captopril treatment. ** Denotes $P < 0.01$.

Correlation

Before captopril. Pre-exercise PA II was positively correlated to PRA ($R = 0.80$, $P < 0.02$) but PAC showed no significant correlation to PA II ($R = 0.66$, $0.1 > P > 0.05$) or to PRA ($R = 0.58$, $0.1 > P > 0.05$). PNA was not significantly related to PRA, PA II or PAC, whereas PA was positively correlated to PRA ($R = 0.76$, $P < 0.05$) and to PA II ($R = 0.74$, $P < 0.05$) but not to PAC ($R = 0.45$, $P > 0.1$).

During exercise there was a direct relation between the increase in the systolic blood pressure (difference between the pre-exercise value and that after 20 min of exercise) and the change in PNA ($R = 0.86$, $P < 0.01$). On the other hand, no significant correlation was found between the blood pressure increase and the changes in PRA, PA II, PAC or PA. Furthermore, no significant relation was recorded between the increase in heart rate and diastolic blood pressure following exercise on the one hand and the changes in PNA, PA, PRA, PA II and PAC on the other hand.

During captopril. Pre-exercise, the decrease in PA II and PAC was positively correlated to the respective values before captopril ($R = 0.98$, $P < 0.001$ and $R = 0.73$, $P < 0.05$). The increase in PRA was also positively related to PRA before captopril ($R = 0.78$, $P < 0.05$). The decrease in diastolic blood pressure showed a significant positive correlation to PRA before captopril ($R = 0.74$, $P < 0.05$) but not to PA II and PAC before captopril ($R = 0.63$, $0.1 > P > 0.05$ and $R = 0.36$, $P > 0.1$, respectively). The decrease in diastolic blood pressure was also significantly related to the captopril-induced increase in PRA ($R = 0.82$, $P < 0.05$) and the decrease in PAC ($R = 0.73$, $P < 0.05$) but not to the decrease in PA II ($R = 0.66$, $0.1 > P > 0.05$).

During exercise neither the increase in systolic or diastolic blood pressure, nor the change in heart rate correlated to the changes in PNA, PA, PA II, PRA or PAC.

Discussion

The findings in the present studies of unaltered exercise-induced increase in plasma catecholamines and heart rate during medication with captopril in spite of a pronounced simultaneous decrease in PA II suggest that angiotensin II does not play a major role for the regulation of sympathetic activity in patients with essential hypertension, neither under basal conditions, nor during short-term physical exercise. Similar results were observed by Hulthén & Hökfelt [17] in an acute study using the converting enzyme inhibitor SQ 20.881 under basal conditions in patients with essential and renovascular hypertension. Unaltered basic PNA

was recently reported also by other authors following captopril in patients with essential hypertension [7–9, 18]. Following head-up tilting Morganti *et al.* [18] found an augmented increase in PNA during captopril. It should, however, be kept in mind that the passive activation of the sympathetic nervous system by tilting is not comparable to the activation induced by almost maximal physical exercise. Nevertheless, all studies presented so far seem to support the view that angiotensin II is not of major importance for the regulation of sympathetic activity in patients with essential hypertension, neither under basal conditions nor in connection with heavy physical exercise. It could be argued that the captopril induced blood pressure reduction, which mainly is due to peripheral vasodilatation, should be associated with a compensatory increase in sympathetic activity analogous with that reported during treatment with hydralazin [19]. Accordingly, the unaltered levels of plasma catecholamines found during captopril could reflect a certain inhibitory effect of captopril on the sympathetic nervous system.

Furthermore, the finding of a direct relationship between the increase in systolic blood pressure and in PNA before medication underlines the dominant role played by the sympathetic nervous system in blood pressure regulation during exercise. Again, the lack of such a correlation during captopril could indicate that captopril has some effect on the regulation of sympathetic tone.

Unaltered blood pressure response to exercise before and during medication despite a pronounced reduction in angiotensin II formation during captopril indicates that angiotensin II is not a major determinant of blood pressure regulation during exercise in hypertensive patients. This view is supported by Fagard *et al.* [20, 21] demonstrating only a minor role for angiotensin II in the pressure elevation during physical exercise in male volunteers by the use of saralasin. Despite a greater stimulation of angiotensin II during exercise in sodium-deplete individuals, the contribution of angiotensin II to the exercise induced rise in pressure was not different from the observation in sodium-replete subjects.

All patients performed the same amount of exercise on captopril as before medication. The achieved mean heart rate level indicates that the work load was near maximal. The perceived exertion rating was the same before and during captopril treatment. Thus, the submaximal work capacity as measured over 20 min in patients with essential hypertension seems not to be reduced by short-term captopril treatment.

The studies demonstrated a close relationship between the reduction in pre-exercise diastolic blood pressure and the pretreatment PRA and probably also to a decrease in angiotensin II, as reflected in reduced plasma aldosterone and increased PRA. These findings are in accordance with the concept that inhibition of angiotensin II formation is an important factor for the captopril induced decrease in diastolic blood pressure under basal conditions.

In conclusion, the present studies support the view that captopril has no profound effect on the activity of the sympathetic nervous system neither under basal conditions nor during heavy physical exercise, in spite of a profound decrease in PA II, in patients with essential hypertension.

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